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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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Plankton and Chemical–Physical Development in Six Swedish West Coast Lakes under Acidic and Limed Conditions

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Increases in pH from 4.5–5.3 to 7–7.6 after liming (1978–85) in six acidified lakes in the west coast area of Sweden caused increased concentration of humic compounds and decreased transparency compared with levels before liming. Concentrations of total P increased markedly whereas those of NO_3-N decreased. The phytoplankton composition changed completely in all lakes shortly after liming, but a species richness similar to that in unacidified lakes was not reached until after 2–5 yr, and in the previously most acidified lakes, several species remained absent. The composition of the phytoplankton indicated increased primary production. The volumetric dominance within the zooplankton shifted generally from *Eudiaptomus–Holopedium* to *Cyclops–Daphnia*. The occurrence of Rotatoria and Cladocera species increased and the composition became similar to that in unacidified lakes 2–5 yr after liming. The biotic development is largely explained by chemical–physical changes such as increased pH, reduced Al concentration, increased P concentration, and increased organic content, particularly humus.

Une augmentation du pH de 4,5–5,3 à 7–7,6, après le chaulage de six lacs acidifiés de la côte ouest de la Suède, a entraîné une augmentation de la concentration des composés humiques et une diminution de la transparence, comparativement aux valeurs mesurées avant le chaulage. La concentration de P total a augmenté de façon marquée, tandis que celle du N sous forme de NO_3 a diminué. La composition du phytoplancton a changé complètement dans tous les lacs peu de temps après le chaulage, mais il a fallu 2 à 5 ans avant d'obtenir une richesse en espèces comparable à celle que lón observe dans les lacs non acidifiés, et dans les lacs qui étaient les plus acidifiés, plusieurs espèces sont demeurées absentes. La composition du phytoplancton a indiqué une augmentation de la production primaire. De manière générale, la dominance volumétrique au sein du zooplancton s'est modifiée, passant d'*Eudiaptomus–Holopedium* à *Cyclops-Daphnia*. La présence des espèces de rotifères et de cladocères a augmenté et, 2 à 5 ans après le chaulage, la composition est devenue semblable à celle que l'on observe dans les lacs non acidifiés. Le développement biotique s'éxplique en grande partie par des modifications chimiques et physiques comme l'augmentation du pH, la réduction de la concentration de l'Al, l'augmentation de la concentration du P et la diminution de la matière organique, et particulièrement de l'humus.

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In Sweden, more than 40% of the total of about 85 000 lakes are considered to be influenced by acidification. The effects appeared earliest in the west coast area where low pH values and deteriorating fish reproduction were already noted during the 1960's (Almer 1972). The alarming situation was confirmed by lake investigations in 1971 (Almer et al. 1974) and in 1976 (Almer et al. 1978), when considerable changes were also found in the plankton composition compared with lakes with neutral pH values.

Strongly acidified lakes with pH values around 5 are frequently characterized by water with high transparency and high concentrations of metals, particularly Al, which has been found to prevent the growth of several plankton species (Hörnström et al. 1984). Aluminum is also assumed to reduce P availability (Broberg 1988), which in turn limits production in waters in the Swedish west coast area. The deficiency of humus and other allochtonous organic supplies further seriously impoverishes the conditions for zooplankton, the food of which in such waters consists almost exclusively of phytoplankton made sparse by extremely low total P concentrations.

After liming and recovery to pH values around 7, the concentrations of Al, Mn, Zn, and Cd decreased (Hasselrot et al. 1984; Wright 1985; Driscoll et al. 1989; Molot et al. 1990a), while the content of organic C gradually increased (Molot et al. 1990a). This change reduced metal toxicity and favoured conditions for flora and fauna. Although the effects of liming on water quality persist, this does not guarantee a normal biotic composition for the future because of slow reinvasion of organisms and because essential nutrients may still be missing, and acidic flushes and reliming act as stress factors.

In Sweden, about 4500 lakes have been limed, which makes this the largest environmental mitigation effort ever undertaken. Regarding long-term changes to the biota, little has been reported from Sweden or other countries. In addition, apart from the finding that pH increases have led to taxonomic changes of phytoplankton (e.g., Yan and Dillon 1984; Larsson 1988), some results are contradictory. Primary production and biovolume were reported by Larsson (1988) to increase whereas Molot et al. (1990b) found changes neither in the chlorophyll concentration nor in the biovolume. Assessments of liming with regard to nutrient concentrations also vary. Wright (1985) reported, for example, an increased P concentration in Norwegian Lake Hovvattn whereas Molot et al. (1990b) found no significant changes in Canadian lakes. Effects on the fauna were

generally considered to result from modified predation (e.g., Henrikson and Oscarson 1984; Evans 1989; Keller et al. 1990), but other possible causes included increased nutrient availability for detritus-living organisms (Raddum et al. 1984) and "improved water quality" (Keller et al. 1990). However, there is a concensus regarding the imbalance of the ecosystem during the first years after liming (e.g., Evans 1989), and that 2 yr is far too short a period for study of the recolonization of the fauna (Keller et al. 1990). In addition, such evaluations are complicated by fluctuating precipitation and thus varying nutrient supply. Factors affecting biological development after liming, e.g., climate, water flow, humus concentration, lime dose, and biotic status, vary strongly among lakes, and consequently, we may expect that the effects of treatment will vary correspondingly. Therefore, long-term studies of lakes of widely differing character are essential to achieve a more general evaluation of the biotic effects of liming. However, apparently no such studies on plankton have been done.

The present study examines liming effects until 1987 in six strongly acidified lakes which have been monitored for up to 9 yr before and 10 yr following treatment. The waters of the studied lakes all had a similar oligotrophic character and were limed in the same way. Therefore, data presentation and discussion concentrate on studies in Lake Tvärsjön. In this paper, three major questions are addressed. Is production-restricting P deficiency relieved solely by liming, or is fertilization required to reestablish the plankton production? How rapidly will inhibited organisms recover, and what is the period of time required for recolonization of lakes by species susceptible to acid conditions? Does liming lead to a plankton community similar to that in unacidified lakes?

Materials and Methods

Study Lakes

Lakes Tvärsjön, Stora Härsjön, Stockasjön, Stora Svansjön, Skällingesjön, and Bossjön are situated in the same area on the Swedish west coast (Fig. 1). The catchment areas are covered by coniferous forest on thin soils, and the absence of agriculture or other sources of nutrients gives the lakes, especially Lake Tvärsjön with long water retention time (Table 1), a marked oligotrophic character.

All the lakes have a dimictic thermal pattern with stratification from June to September, where the epilimnion has a depth of about 10 m with a summer temperature of 16–21°C. Lake Tvärsjön from which we present more detailed data has steep shores covered by stone and gravel. The lake has a very restricted littoral area, and the water vegetation is sparse.

Since 1979, studies have also been performed in neutral Lake Stora Färgen (pH 6.7–7.0), which was used as a reference for the degree of recovery in limed lakes. We had no control over liming procedures, but because the liming period (Table 1) differed among the lakes and ranged from 1977 to 1984, some acidic and unlimed lakes could also serve as reference for degree of change after liming, up to 1985.

FIG. 1. Location of six acidified lakes and one neutral lake (Stora Färgen) studied on the west coast of Sweden.

The plankton of the limed lakes was also compared with that of 20 lakes in the same area which had resisted acidification. When selecting these lakes, it was necessary to go back to the 1970's, when they were unlimed. These lakes were investigated in surveys in 1971, 1972 (Dickson et al. 1973, 1975), and 1976 (Hörnström and Ekström 1983). To ensure similarity of lakes, those with a water colour of 5–20 mg Pt/L and a Secchi disc depth of 4.5–7 m were selected. In order to check the pH effect on phytoplankton species distribution, the lakes were separated into two pH groups with ranges of 6.2–6.5 and 6.8–7.4. Only the ten lakes of the latter pH group were used as references in the zooplankton investigation.

Liming

All the studied lakes were limed during 1982–84, except Lake Stora Härsjön which was treated in 1977–78. Lakes Boss-

TABLE 1. Morphometric characteristics of the study lakes.

Lake	Surface area (ha)	Altitude above sea level (m)	Max. depth (m)	Mean depth (m)	Retention time (yr)	Liming period
Stora Färgen	600	61	48	16	4.8	—
Tvärsjön	100	100	34	19.5	8	June 1982
Stora Härsjön	245	90	42.5	15	3.5	Winter 1977–78
Stockasjön	90	103	40	12	5.6	May 1983
Stora Svansjön	86	101	16	5.1	2.9	Spring 1982–84
Bossjön	45	126	21	7	1	Autumn 1982–84
Skällingesjön	30	70	26	10.5	8.3	August 1984

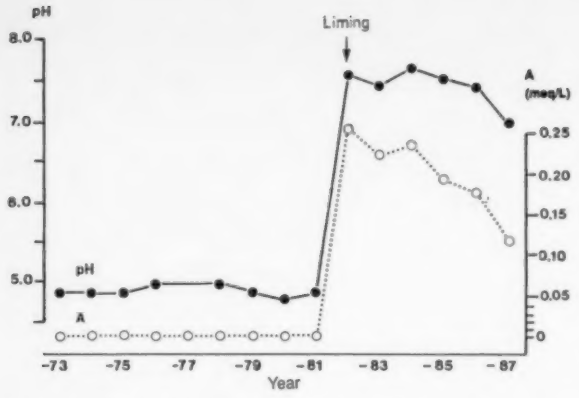

FIG. 2. pH (solid circles) and alkalinity (open circles) in Lake Tvärsjön, 1973–87 (August values).

jön and Stora Svansjön were relimed in 1984. In Lake Tvärsjön the dosage in June 1982 was 558 tonnes of lime. Limestone powder (grain size 0–0.5 mm) was spread over deep areas of the lake, along the shorelines, and over the inlet areas. The treatment increased epilimnic pH values from 4.7 to 7.6 (Fig. 2) (in other lakes from 4.5–5.3 to 7.0–7.5) in a few months.

Physical and Chemical Sampling

The physical–chemical studies were concentrated during the stratification period of July–August, since this offered the most stable conditions and consequently the most representative samples. The sampling was conducted in the middle of the lakes at a depth of 0.5 m, without using a sampler, to avoid contamination. As the levels of total P were very low, utmost care was necessary. While moving the boat forwards, a 250-mL, acid-washed polyethylene flask was lowered from the bow of the boat vertically into the water, after which the flask was turned forwards and filled. The flask was rinsed three times using the above procedure before the final sample was taken. By this means the error caused by contamination was minimized and the P concentrations of the three replicates, sampled every 100 m, were usually identical (analytical accuracy ± 0.5 µg/L). The samples were preserved with 4 M H_2SO_4 (1 mL/100 mL). Samples for other analyses were not acidified. The physical and chemical parameters analysed were Secchi disc depth, visual water colour, colour (milligrams Pt per litre), temperature (using a thermistor thermometer), dissolved organic carbon (DOC), pH, alkalinity, conductivity, Na, K, Mg, Ca, SO_4, Cl, total P (tot-P), NO_3-N, total N (tot-N), and total Al (tot-Al).

The pH was determined using a radiometer refillable glass electrode, while alkalinity was determined by titration with hydrochloric acid to pH 5.4, with N_2 bubbling to eliminate produced CO_2. Ca, Na, and Mg were determined by flame atomic absorption spectroscopy (AAS) and SO_4 by titration with barium chloride and Thorine indicator with photometric endpoint determination. Cl was analyzed by potentiometric titration. Total P was determined by using an autoanalyzer and by converting organic P compounds and inorganic polyphosphates to orthophosphate by boiling, 30 min under pressure, with peroxodisulphate in acid solution. The orthophosphate formed was determined after reaction with ammonium molybdate forming phosphomolybdic acid, which was then reduced by ascorbic acid. The coloured molybdenum blue complex formed was measured at 880 nm. Nitrate was analyzed by reduction to NO_2 on a copper/cadmium column, after which the NO_2 was determined by diazotation and coupling with *N*-(1-naphthyl)ethylendiamine, followed by a colorimetric measurement. Total N was analyzed by boiling with peroxodisulphate in basic solution. All nitrogen compounds were oxidized to NO_3, which was then determined as above. All the analyses were performed according to the Swedish standard methods (Svensk Standard, Series SS 0281, Box 3295, S-10366 Stockholm, Sweden).

Sampling of Plankton

The study of plankton was started in 1971 in Lakes Stora Härsjön, Skällingesjön, Bossjön, and Stockasjön and in 1973 in Lakes Stora Svansjön and Tvärsjön. Except in Lake Tvärsjön the sampling was not done every year, until 1980. In Lake Tvärsjön the phytoplankton was sampled every second week during April–November in 1973 and 1977. At that time the analyses showed no marked fluctuations in species composition, which changed only gradually. (The dinophyceans dominated throughout June–August, while some chlorophyceans and chrysomonads were also frequent in May and September–October). On the basis of this result the sampling during 1974–76 and 1978–87 was conducted only in mid-May, July, and August at depths of 0.5, 5, 10, and 15 m. Preliminary sampling of phytoplankton at different sites in the lake showed no marked difference in species composition or abundance, thus indicating similar water quality. In addition the surface area of Lake Tvärsjön is only 1 km^2 and the lake has no separate basins. Therefore, all the sampling was done at the same location in the middle of the lake. The phytoplankton samples, collected by a Ruttner sampler, were preserved with Lugol's solution (Willén 1974). One hundred millilitres was analyzed under an inverted microscope using magnifications of 25, 100, and 400. The total number of species was recorded and the estimated abundances classed as follows 1 = <500, 2 = 500–5000, 3 = 5000–25 000, 4 = 25 000–100 000, and 5 = >100 000 ind./L, except for larger forms (>6000 μm^3), which were counted with the lowest magnification, where corresponding numbers were <20, 20–200, 200–1000, 1000–5000, and >5000 ind./L. Phytoplankton biovolumes were determined according to Willén (1974).

The zooplankton was sampled at the same time as the phytoplankton with nets (mesh sizes of 75 and 25 µm) which were hauled from a depth of 13 m (i.e., 1–3 m above the bottom at the sampling station) to the surface. The nets were cylindrical with a conical end. The 75-µm net had a diameter of 0.27 m and a total length of 0.75 m, while corresponding values for the 25-µm net were 0.16 and 0.55. The sampling was done at similar depth in the other lakes, except for Lake Stora Svansjön where the sampling depth was only about 7 m. The samples were preserved in the same way as those of phytoplankton, and the frequency of each species was estimated using the same scale (1–5) as for the phytoplankton. The biovolume of the zooplankton was calculated by a slight centrifugation (2000 rpm for 5 min) in graduated cylinders.

Results

Physical and Chemical Conditions

The discharge, measured at River Rolfsån (nearest hydrological station, situated 32 km southwest of Lake

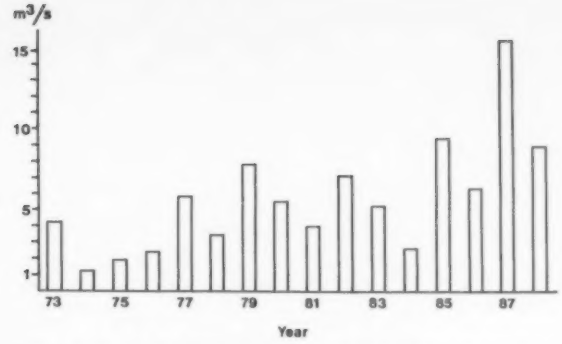

FIG. 3. Mean discharge of the River Rolfsån, May–September.

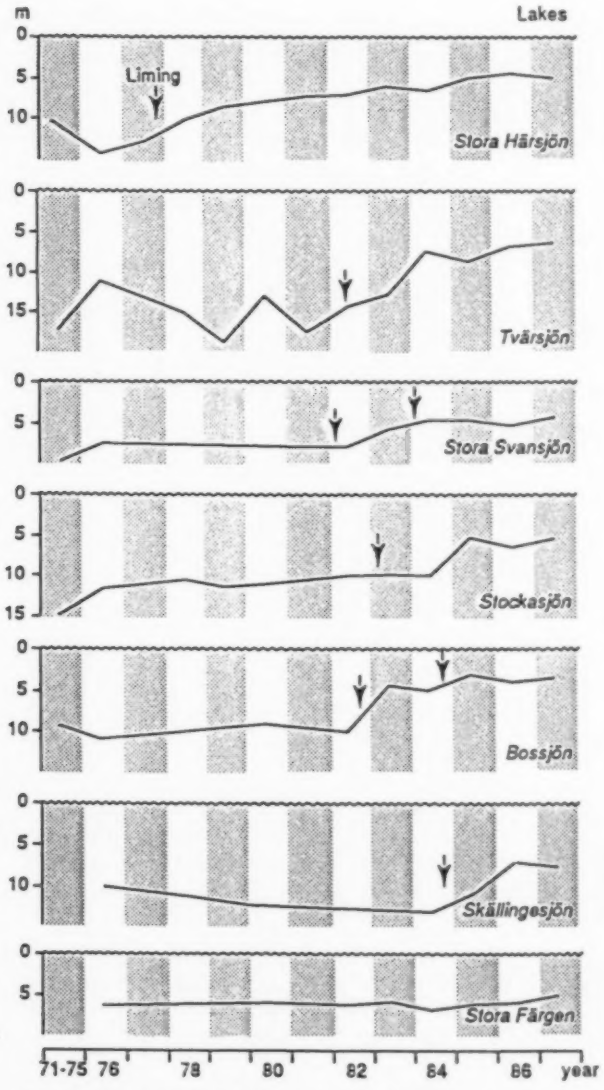

FIG. 4. Secchi disc depth before and after liming (arrows) in the acidified lakes and in unacidified Lake Stora Färgen.

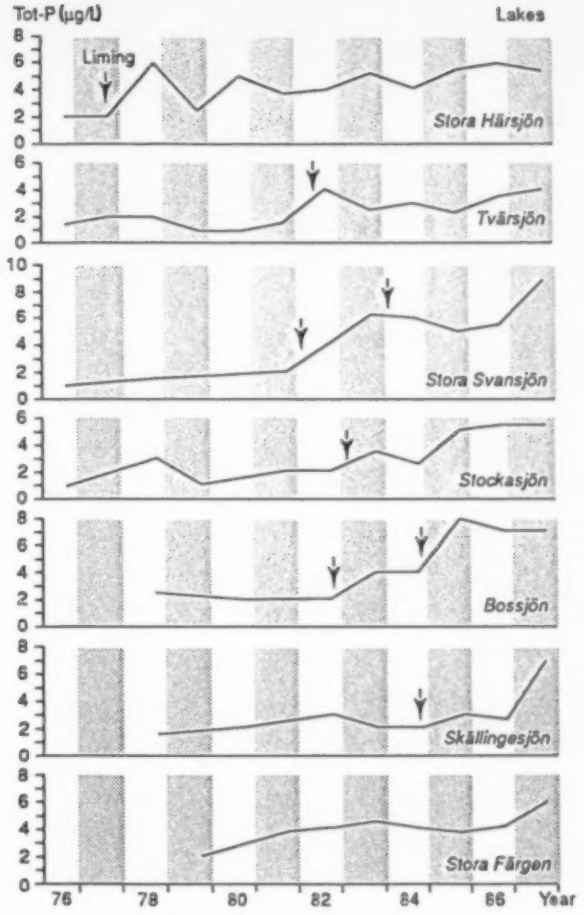

FIG. 5. Levels of total P in Lakes Tvärsjön, Stora Svansjön, Stockasjön, Bossjön, Stora Härsjön, and Skällingesjön before and after liming (arrows) and in unacidified Lake Stora Färgen.

Tvärsjön), varied widely (Fig. 3). This, however, did not affect the water quality of the acidified lakes before liming. Throughout, the Secchi disc depths were high (Fig. 4) and the visual colour was blue – emerald green, indicating absence of humic compounds (colour <5 mg Pt/L).

In contrast with the acidified lakes, the water of Lake Stora Färgen (unacidified reference lake) was yellow–green (colour 5–20 mg Pt/L) and less transparent, with Secchi disc depths varying between 5.5 and 7.1 m (Fig. 4). As in other lakes of the area, the concentrations of NO_3 were high during the period of study but with 23–35% lower levels in summer (300–475 µg/L) than in spring. The P concentration remained stable around 4 µg/L until 1987, when there was an increase to 6 µg/L (Fig. 5). The levels of Al were stable at about 20 µg/L in summer. Other chemical data from Lake Stora Färgen are presented in Table 2.

Before liming of Lake Tvärsjön, the concentration of DOC was ≤1.4 mg/L and pH was 4.7–4.9 (Fig. 2), while the Secchi disc depth varied between 10 and 18 m (Fig. 4). In relation to climatic changes and the concentration of the major constituents (Table 2), the conductivity varied between 7.3 and 7.8 mS/m.

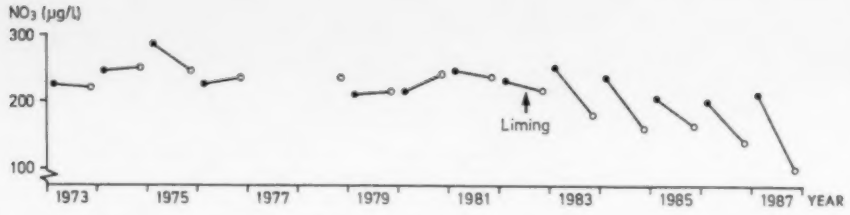

FIG. 6. Concentration of NO_3 in May (solid circles) and August (open circles) Lake Tvärsjön.

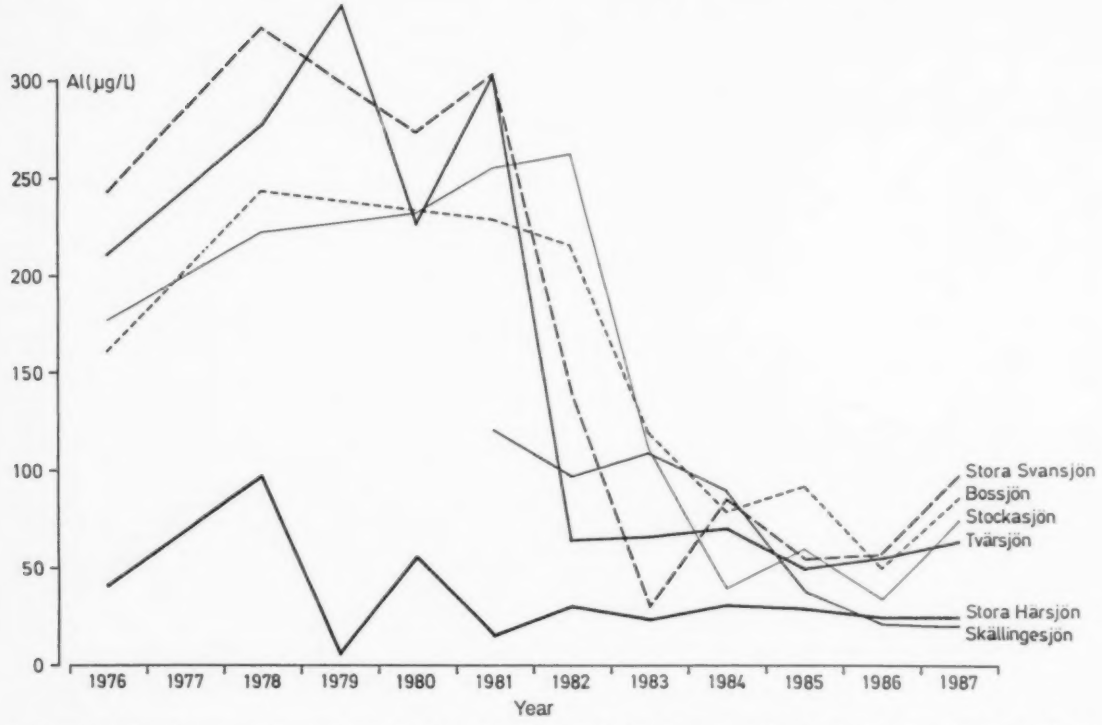

FIG. 7. Average epilimnic concentrations of Al (July–August) in Lakes Tvärsjön, Stora Svansjön, Stockasjön, Bossjön, Stora Härsjön, and Skällingesjön, limed in June 1982, spring 1982, May 1983, autumn 1982 and 1984, winter 1977–78, and August 1984, respectively.

TABLE 2. Chemical characteristics of Lake Tvärsjön limed in 1982 and unacidified Lake Stora Färgen.

	Tvärsjön		Stora Färgen,
	1974–81	1982–87	1979–87
Conductivity (mS/m)	7.3–7.8	9.4–7.5	9.4–11.3
Alkalinity (meq/L)	<0.01	0.26–0.12	0.06–0.08
pH	4.7–4.8	7.0–7.6	6.7–7.0
Na (meq/L)	0.27–0.29	0.24–0.31	0.32–0.35
K (meq/L)	0.02	0.02	0.02–0.03
Mg (meq/L)	0.09–0.11	0.09–0.11	0.14–0.15
Ca (meq/L)	0.12–0.14	0.42–0.30	0.27–0.29
SO_4 (meq/L)	0.25–0.29	0.20–0.25	0.24–0.30
Cl (meq/L)	0.27–0.30	0.26–0.30	0.36–0.40
Cd (µg/L)	0.2–0.3	<0.05	<0.05
Zn (µg/L)	20–30	<4	<4
Mn (µg/L)	200–230	<4	<4
NO_3-N (µg/L)	215–245	140–180	300–475
Tot-N (µg/L)	330–480	290–320	580–620
Colour (mg Pt/L)	<5	5–10	5–20

During the period 1973–81, total P was only 1–2 µg/L (Fig. 5), while NO_3 varied between 210 and 285 µg/L (Fig. 6). Except in 1975 and 1981 the NO_3 level was not lowered during summer. High levels of NO_3 are typical for all the studied lakes.

After liming of Lake Tvärsjön in June 1982 the alkalinity had increased to 0.22 meq./L and the pH value to 7.4 by mid–July. Thereafter, until 1987 the values gradually decreased to about 0.12 and 7.0, respectively (Fig. 2). The level of Ca increased from 0.10 to 0.39 meq./L and the conductivity from 7.3–7.8 to 8.1–9.4 mS/m.

As a result of the pH change the concentration of Al (epilimnic values) decreased in all the lakes (Fig. 7). This was exemplified in Lake Tvärsjön where the value fell from 331 µg/L in June to 113 µg/L in July and 66 µg/L in August. The epilimnic values in July–August 1983–87 (after liming) were 50–75 µg/L, but somewhat higher in May.

The DOC levels in Lake Tvärsjön increased from 0.4–1.4 to 2.3–4.65 mg/L after the treatment, and the water colour increased from <<5 to 5–15 mg Pt/L. As a result, the transparency decreased as in the other limed lakes (Fig. 4) and

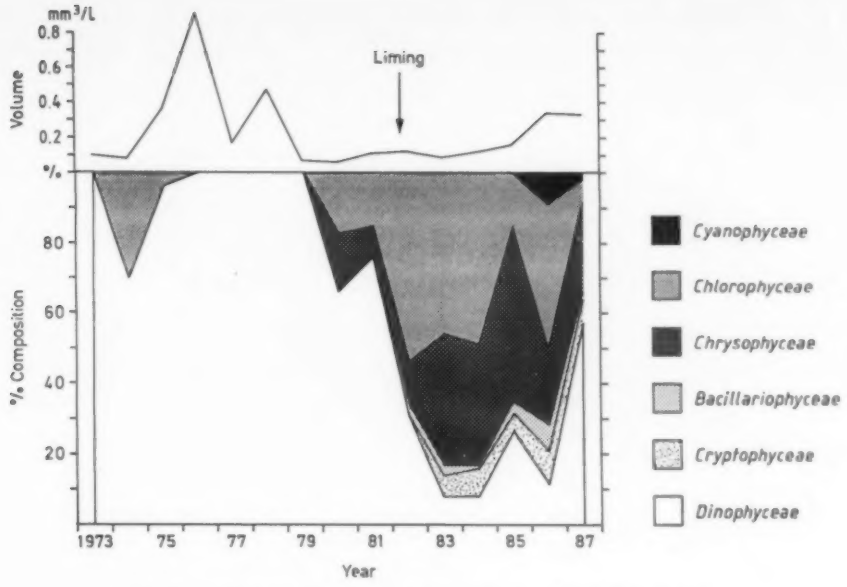

FIG. 8. Phytoplankton volume and percent composition in Lake Tvärsjön.

varied between 6.7 and 9 m in 1984–87 compared with 10–18 m before liming.

After liming of Lake Tvärsjön in June 1982, the concentration of total P increased from 1–2 to 4 μg/L. Concentrations decreased temporarily in 1983 but on average were significantly ($P = 0.004$) higher after liming (3.25 compared with 1.6 μg/L) and reached a maximum in 1987. The other lakes followed much the same course (Fig. 5).

In 1983–87 the concentration of NO_3 in May was little changed by liming. The August values, however, were markedly lowered from 210–250 to 180–90 μg/L (Fig. 6), with the minimum value in 1987. In Lake Bossjön, where the highest levels of P occurred (Fig. 5), NO_3 concentration decreased to around the detection limit (±5 μg/L) in 1985–86. During 1982–87 the deposition of NO_3 from the air increased markedly in the west coast area (Lövblad 1989), indicating that the decrease in Lake Tvärsjön was a result of liming and increased N assimilation by the primary producers.

Phytoplankton

The phytoplankton of Lake Stora Färgen (reference lake) was highly diversified and, on average, more than 70 taxa were recorded per sample during 1979–87. Diatoms, generally *Cyclotella comta*, constituted the bulk of the biomass whereas chlorophyceans were quantitatively unimportant. The total algal average volume varied in July–August between 0.3 and 0.9 mm^3/L, with maximum values in 1982 and 1985.

In Lake Tvärsjön, there was no marked seasonal succession of phytoplankton during the acidic period. Thus, in 1973 and 1977 (when the lake was investigated every other week) the typical acid lake dominants *Gymnodinium uberrimum* and *Peridinium inconspicuum* occurred from spring to September, as also did the chlorophyceans *Chlamydomonas* sp., *Monoraphidium dybowskii*, *Oocystis submarina*, and *Tetrastrum komarekii*. During spring and autumn, the Chrysophyceae group was most prominent both in number of species and in share of biomass. The characteristic species were *Dinobryon crenulatum* and *D. sociale* var. *americanum*. In some periods, real acidophilic species, *Dinobryon pediforme* and *Pseudokephyrion gibbosum*, were present. *Dinobryon cylindricum* var. *alpinum* and *Peridinium aciculiferum* were recorded only in May, and surveys in other lakes in the area, acidified or not, indicated that they were cold-water forms.

The number of phytoplankton taxa in Lake Tvärsjön recorded in each sample varied in May between 18 and 31, the highest value being in 1981. In clear and sunny weather the phytoplankton volume consisting of motile flagellates had its maximum at a depth of about 10 m, indicating highly transparent water.

During June–August the standing stock of phytoplankton consisted mainly of the dinoflagellates *Peridinium inconspicuum*, *Gymnodinium uberrimum*, and *Amphidinium* sp. In 1980–81, however, small chlorophyceans were frequent. As in spring, the volumetric distribution of plankton (dinophyceans) according to depth was light dependent, and volumes as high as about 2 mm^3/L were recorded in single samples. The average volume for the 0.5- to 15-m water column, however, never exceeded 0.4 mm^3/L, except in 1976 and 1978, when the volumes were at a maximum (Fig. 8). Before liming, the average number of taxa recorded in summer was below 15, except in 1981, when there was a maximum of 18 (Fig. 9).

In July and August 1982, after liming, the species number of phytoplankton was still low and the standing stock was decreased. *Gyromitus cordiformis* and related forms of the group Pyramimonaceae, not occurring before the treatment, dominated, especially in the hypolimnion. Other new species were *Cosmarium* sp. and *Synedra nana*.

The taxonomic composition in spring 1983 was similar to that before liming, while in August, several new species, especially *Sphaerocystis schroeterii* (Table 3), occurred and made up the bulk of the biomass together with the remaining Pyramimonaceae. It was not until 1985, however, that the species richness increased markedly (Fig. 9). A marked difference

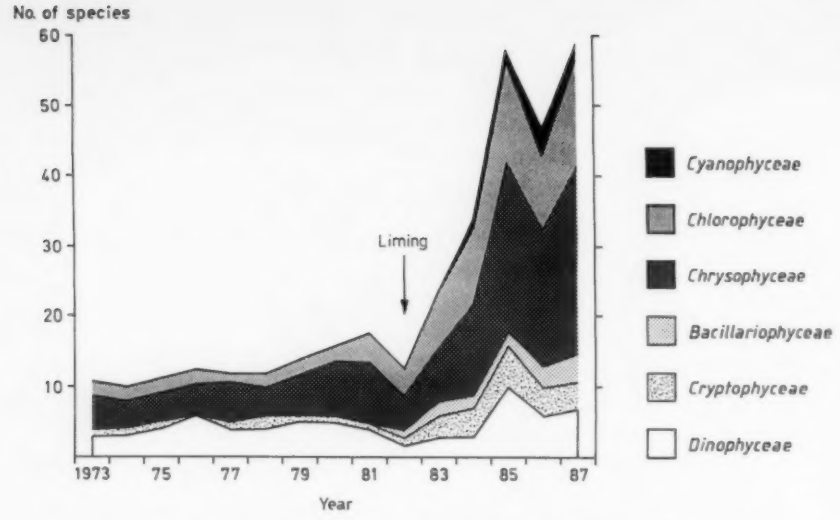

FIG. 9. Number and distribution of phytoplankton species in Lake Tvärsjön.

compared with the acidic period was the invasion of the groups Cyanophyceae and Bacillariophyceae.

The total volume of phytoplankton in Lake Tvärsjön remained relatively low during 1982–84 (Fig. 8) but, like the species number, it increased and was 0.22–0.34 mm^3/L in 1986–87. On average, it was, however, lower than before liming. Except for the first years after liming, all the common oligotrophic groups contributed to the biomass, and the characteristic acid-tolerant dinophyceans *Peridinium inconspicuum* and *Gymnodinium uberrimum* were mostly replaced by *Ceratium hirundinella*, *Peridinium willei*, and *P. goslaviense*.

The phytoplankton volumes in summer before liming in lakes Stora Härsjön, Stockasjön, Stora Svansjön, Bossjön, and Skällingesjön varied strongly. On average, volumes increased after liming (Table 4). Low sampling frequency and high variance, however, partly made these results less significant. Only in Lakes Stora Svansjön ($P = 0.016$) and Bossjön ($P = 0.006$) did the volumes significantly increase.

As in Lake Tvärsjön, *Peridinium inconspicuum* and *Gymnodinium uberrimum* were often dominants, especially in Lake Stockasjön, whereas chrysomonads were most common in Lakes Stora Svansjön and Skällingesjön. A few years after liming, the species composition of phytoplankton had changed in all the limed lakes. The typical dinophyceans were mainly replaced by a mixed assemblage, including all common groups. The frequency of those chrysomonads which occurred under acidic conditions decreased in favour of new species with higher trophic requirements. Thus, *Dinobryon crenulatum* and *D. sociale* var. *americanum* were replaced by *D. divergens*, *D. bavaricum*, and other forms. The species richness increased markedly, and 2–4 yr after liming an average of 47 taxa (40–54) per sample was recorded in summer compared with 15 (14–17) before treatment.

The comparison between unacidified reference lakes with different pH ranges shows that pH value, also in unacidified lakes, is important to the species composition. Thus, *Aphanothece ellipsoidea* (Cyanophyceae) and *Cyclotella spp* (Bacillariophyceae), on the one hand, occurred only at high pH whereas some desmids (*Staurastrum/Staurodesmus*), on the other hand, occurred more frequently among lakes with a low pH range. However, among 53 taxa, common in the reference lakes during 2 yr, several species, also within the high pH range, cyanophyceans and diatoms, were still missing in many limed lakes 3–4 yr after liming (Table 5). Only in Lake Stora Härsjön, which was somewhat less acidified, did a normal diatom plankton community develop.

Zooplankton

A total of 28 species of zooplankton was recorded in Lake Stora Färgen (reference lake) in 1981–87, including 15 rotatorians, nine cladocerans, and four copepods (ciliates not included). The number of species recorded at each sampling was in the range 16–21 (Table 6). The most frequent species within the Rotatoria group were *Conochilus unicornis*, *Kellicottia longispina*, *Keratella cochlearis*, *Polyarthra remata*, and *P. vulgaris*. The Cladocera group was dominated mainly by *Holopedium gibberum*, *Bosmina longispina*, and *Daphnia galeata* whereas Copepoda consisted of *Cyclops* spp. and *Eudiaptomus gracilis*. There were no marked temporal changes in the zooplankton composition in Lake Stora Färgen during the period studied.

The zooplankton volume in Lake Stora Färgen, which was made up mainly of cladocerans and copepods, was on average 1.06 mm^3/L and in the range 0.53–1.74 mm^3/L, with the maximum value in 1983 (Table 6).

As the zooplankton composition varied among lakes the comparison with only Lake Stora Färgen is not entirely appropriate. Therefore, the reference material also includes data from 10 lakes in lake surveys in 1971 and 1976 (see Materials and Methods). These lakes were in the pH range 6.8–7.4. The most common species of zooplankton in the reference lakes were *Asplanchna priodonta*, *Conochilus unicornis*, *Kellicottia longispina*, *Keratella cochlearis*, *Polyarthra remata*, and *P. vulgaris* (Rotatoria), *Bosmina longispina*, *Daphnia cristata*, *Diaphanosoma brachyurum*, and *Leptodora kindtii*, (Cladocera), and *Cyclops* spp. and *Eudiaptomus gracilis* (Copepoda).

In Lake Tvärsjön the groups Cladocera and Copepoda were

TABLE 3. Occurrence (+) in Lake Tvärsjön of 41 phytoplankton taxa recorded every year in reference Lake Stora Färgen during 1979–87.

	Before liming		After liming		
	1977–78	1979–81	1982–83	1984–85	1986–87
Cyanophyceae					
Anabaena flos-aquae					
Aphanocapsa spp.					
Aphanothece ellipsoidea					+
Chroococcus limneticus					+
Gomphosphaeria lacustris					
Merismopedia spp.				+	+
Chlorophyceae					
Botryococcus braunii			+	+	+
Chlamydomonas spp.	+	+	+	+	+
Crucigenia tetrapedia					
Elakatothrix genevensis			+	+	+
Monoraphidium dybowskii	+	+	+	+	+
Monoraphidium griffithii				+	
Nephrocytium agardhianum				+	+
Oocystis spp.	+	+	+	+	+
Pyramimonaceae spp.			+	+	+
Scenedesmus denticulatus			+	+	+
Sphaerocystis schroeterii			+	+	+
Staurastrum pseudopelagicum					
Staurodesmus sellatus					
Chrysophyceae					
Chrysochromulina sp.			+	+	+
Chrysococcus spp.			+	+	+
Dinobryon bavaricum				+	+
Dinobryon borgei				+	+
Dinobryon divergens				+	+
Dinobryon crenulatum	+	+	+	+	+
Mallomonas spp.				+	+
Ochromonas/Chromulina spp.	+	+	+	+	+
Pseudopedinella sp.			+	+	+
Spiniferomonas spp.			+	+	+
Stichogloea doederleinii					+
Bacillariophyceae					
Asterionella formosa					+
Cyclotella comta				+	+
Tabellaria fenestrata					+
Tabellaria flocculosa		+	+	+	+
Cryptophyceae					
Cryptomonas erosa	+	+	+	+	+
Katablepharis ovalis			+	+	+
Rhodomonas minuta			+	+	+
Dinophyceae					
Ceratium hirundinella				+	+
Gymnodinium uberrimum	+	+	+	+	+
Peridinium inconspicuum	+	+	+	+	+

composed of only four species during 1974–81 before liming, *Bosmina longispina*, *Holopedium gibberum*, *Cyclops scutifer*, and *Eudiaptomus gracilis*, while nine species of rotifers were recorded (Table 7). *Holopedium*, *Bosmina*, and *Eudiaptomus* were common throughout the season whereas *Cyclops* was abundant only in spring. Among the most common rotifers, *Collotheca* sp. and *Polyarthra remata* were frequent both in May and July–September, while *Asplanchna priodonta* generally peaked in August. These species comprised the main part of the zooplankton volume in Lake Tvärsjön, which was generally low prior to liming, but with a high level in the last acid season (Fig. 10).

The number of species of zooplankton in the acidified lakes was markedly low (Table 6) compared with the reference lakes. However, concerning all the acidified lakes, only *Gastropus stylifer*, *Ploesoma truncatum*, *P. hudsoni*, and *Trichocerca* spp. (Rotatoria) and *Daphnia* spp. (Cladocera) were missing before liming.

Quantitatively dominant crustacean species found in all the lakes included *Bosmina longispina*, *Eudiaptomus gracilis*, and

TABLE 4. Mean phytoplankton volume (mm^3/L) in the study lakes, July-August.

Lake	Before liming			After liming		
	$\bar{X} \pm SD$	Range	n	$\bar{X} \pm SD$	Range	n
Tvärsjön	0.27 ± 0.31	0.06–0.92	9	0.19 ± 0.18	0.09–0.34	6
Stora Härsjön	0.22 ± 0.17	0.16–0.34	4	0.32 ± 0.33	0.15–0.48	10
Stockasjön	0.30 ± 0.31	0.04–0.76	7	0.38 ± 0.18	0.18–0.45	4
Stora Svansjön	0.33 ± 0.32	0.06–0.90	7	0.66 ± 0.27	0.32–1.0	5
Skällingesjön	0.19 ± 0.21	0.10–0.34	7	0.30 ± 0.24	0.08–0.47	3
Bossjön	0.15 ± 0.19	0.10–0.27	4	0.56 ± 0.26	0.30–0.72	5
Stora Färgen	0.43 ± 0.27	0.24–0.89	9	—	—	—
Unacidified reference lakes	0.45 ± 0.20	0.20–0.58	10	—	—	—

occasionally *Holopedium gibberum* in Lakes Stockasjön and Stora Härsjön. Abundant rotatorians were *Asplanchna priodonta*, *Keratella cochlearis*, and *Polyarthra remata* in Lakes Bossjön, Skällingesjön, and Stockasjön, while there was no clear dominance in Lakes Stora Svansjön and Stora Härsjön.

After liming, the species composition changed markedly in all the acid lakes. Thus, the number of rotatorian species was 1.5–3 times higher than before liming (Table 8). New Cladocerans appeared, among which the daphnids (*Daphnia cristata*, *D. longispina*, and *D. cucullata*) were most prominent, together with *Ceriodaphnia quadrangula*, which was also quantitatively important. The number of Copepoda species changed little, but the frequency of Cyclops increased markedly in three of the lakes shortly after liming.

The new zooplankton composition developed slowly. Thus, in Lake Tvärsjön the liming, in June 1982, first markedly reduced abundance and diversity of the zooplankton community. In July, shortly after the treatment, no rotifers were recorded, and there was only a sparse occurrence of *Cyclops* and *Bosmina*. The only frequent species was *Eudiaptomus gracilis*. It was not until 1983 that the diversity increased markedly (Table 7) and a species previously not recorded, *Ceriodaphnia quadrangula* (Cladocera), occurred. The immigration of new species continued. In 1984–85 the cladocerans *Daphnia cristata* (in spring), *D. longispina*, and *Diaphanosoma brachyurum* appeared, and in 1986, *Leptodora kindtii*, *Conochilus unicornis*, and *Synchaeta* sp., and *Cyclops scutifer*, which in summer only had a sparse occurrence before liming, became very common whereas *Eudiaptomus* decreased.

A comparison between the unacidified reference lakes and the limed lakes indicates that the latter, 3–4 yr after liming, have a zooplankton composition rather like that in the reference lakes (Table 8). For instance, *Daphnia cristata*, which was absent before liming, was present only in 17% of the limed lakes 1–2 yr after liming, but 3–4 yr after liming, it occurred in 80% of the lakes; it was also present in 80% of the reference lakes. Among 14 species which occurred in at least 50% of the reference lakes, all occurred in 40–100% of the limed ones, except *Trichocerca* spp. However, some species, e.g., *Collotheca* sp., *Gastropus stylifer*, *Synchaeta* sp., *Ceriodaphnia quadrangula*, and *Polyphemus pediculus*, occurred in >50% of the limed lakes 3–4 yr after liming, but in <30% of the reference lakes. Altogether the total number of species in limed lakes 1–2 yr after liming was 9–20 ($\bar{X}$ = 13.7) and 3–4 yr after was 15–21 ($\bar{X}$ = 18.2). The latter value was similar to species richness in the reference lakes, where 13–21 ($\bar{X}$ = 16.4) species were recorded.

During the acidic period, there was a relationship between the distribution of the phyto- and zooplankton in all the acidified lakes. Thus, *Peridinium* and *Gymnodinium* were associated with *Asplanchna*, *Polyarthra*, and *Bosmina* in 1973 and 1976–79, while *Holopedium* and *Eudiaptomus* were dominants when small chlorophyceans and chrysophyceans were frequent in 1974 and 1980–81. After liming, however, the diversity of both phyto- and zooplankton was markedly increased and it was not possible to distinguish relationships between these groups.

The biovolume of zooplankton in Lake Tvärsjön decreased markedly shortly after liming (Fig. 10). The volume, however, again increased in 1983, and the average level (July–August) over the 6 yr after liming was 0.99 mm^3/L compared with 0.55 mm^3/L in 1975–81 (Table 6). The increase was, however, not significant.

The total average zooplankton volumes in the other acidic lakes varied before liming between 0.12 mm^3/L in Lake Bossjön and 0.96 mm^3/L in Lake Skällingesjön (Table 6). After liming the values were higher throughout than before treatment and were on average from 0.77 mm^3/L in Lake Stora Härsjön up to 1.58 mm^3/L in Lake Bossjön. However, only in the latter lake, where P levels and phytoplankton volumes were also high, was the increase significant (P = 0.007).

Discussion

As in other liming investigations (Dickson 1980; Hasselrot et al. 1984; Wright 1985), the increased pH rapidly reduced the concentration of Al (Fig. 7). As a result, the water colour, unaffected by the varying runoff before liming, gradually increased and changed from Mediterranean blue to distinctly green. At the same time, the transparency decreased. Considering all the studied lakes, we observed that the colour changed to green to yellow to brown, corresponding to water colours 5–50 mg Pt/L. The transparency of the waters, which in oligotrophic lakes mainly depends on the concentration of humic material measured as water colour (Fig. 11), decreased from 7–18 to 4–10 m (Fig. 4). The colour of humus compounds is little affected by pH values in the range 4–8 (Gjessing 1978). It thus seems probable that the optical change in Lake Tvärsjön was caused generally by increased concentration of humic compounds, previously precipitated by Al (Almer et al. 1978; Hörnström et al. 1984). An indication of this is also the increased DOC levels, also recorded by Hasselrot et al. (1984), Wright (1985), and Buckaveckas and Driscoll (1991).

Shortly after the liming, there was a temporary increase in the level of P in Lake Tvärsjön (Fig. 5), probably related to the

TABLE 5. Percent occurrence of 53 phytoplankton taxa, recorded at two investigations in 20 unacidified lakes, grouped into pH ranges 6.2–6.5 (A) (n = 10) and 6.8–7.4 (B), compared with their occurrence in six acidified lakes, during years 1–2 (C) and 3–4 (D) after liming. Only taxa recorded in ≥50% of either reference group are included.

	Percentage of reference lakes		Percentage of limed lakes	
Taxon	A	B	C	D
Cyanophyceae				
Anabaena flos-aquae	80	80	0	17
Aphanocapsa spp.	0	60	0	0
Aphanothece ellipsoidea	0	70	0	17
Chroococcus limneticus	60	60	0	17
Gomphosphaeria lacustris	50	70	0	17
Gomphosphaeria naegeliana	10	50	0	0
Merismopedia tenuissima	80	50	0	0
Chlorophyceae				
Botryococcus braunii	80	100	33	33
Chlamydomonas spp.	90	70	83	100
Cosmarium spp.	80	80	67	50
Crucigenia tetrapedia	90	70	0	0
Elakatothrix genevensis	100	100	67	100
Gleocystis planctonica	70	40	0	0
Monoraphidium dybowskii	100	100	33	83
Monoraphidium griffithii	70	30	0	17
Oocystis spp.	100	90	67	50
Pyramimonaceae spp.	20	70	33	50
Scenedesmus spp.	80	90	50	100
Sphaerocystis schroeterii	70	80	17	33
Staurastrum anatinum	50	20	0	0
Staurastrum longipes	50	20	0	0
Staurodesmus extensus	70	10	0	0
Staurodesmus sellatus	70	70	0	0
Willea irregularis	60	40	0	0
Chrysophyceae				
Bitrichia chodatii	70	90	83	67
Chromulina/Ochromonas	80	80	100	50
Chrysochromulina parva	20	70	50	50
Chrysococcus spp.	80	80	83	100
Dinobryon bavaricum	80	40	33	50
Dinobryon borgei	90	70	0	33
Dinobryon crenulatum	80	90	100	67
Dinobryon divergens	30	50	17	67
Dinobryon suecicum	20	60	0	17
Kephyriopsis entzii	50	40	17	50
Mallomonas caudata	70	50	0	33
Mallomonas spp.	80	60	33	67
Prymnesiaceae spp.	50	20	67	83
Spiniferomonas spp.	80	20	17	67
Stichogloea doederleinii	90	90	0	0
Uroglena spp.	50	50	17	83
Bacillariophyceae				
Asterionella formosa	20	70	0	17
Cyclotella spp.	0	90	17	33
Melosira distans var. *alp.*	20	50	0	0
Rhizosolenia longiseta	40	50	0	17
Tabellaria fenestrata	40	60	0	33
Cryptophyceae				
Cryptaulax sp.	50	30	33	83
Cryptomonas spp.	100	70	100	100
Katablepharis ovalis	80	90	100	100
Rhodomonas minuta	90	100	100	100
Dinophyceae				
Ceratium hirundinella	0	100	0	50
Gymnodinium uberrimum	60	70	67	67
Gymnodinium spp.	80	100	67	100
Peridinium inconspicuum	60	60	83	100

TABLE 6. Mean and total number and volume (mm^3/L) of zooplankton in the study lakes, July–August.

	Before liming			After liming		
	$\bar{X}$ ± SD	Range	*n*	$\bar{X}$ ± SD	Range	*n*
			Stora Svansjön			
Rotatoria	1.5 ± 0.9	0–2	4	6 ± 1.9	3–8	5
Cladocera	1 ± 0	1		3.4 ± 1.6	1–5	
Total	4 ± 0	4		9.8 ± 3.2	6–14	
Volume	—	—	—	0.78 ± 0.29	0.36–1.2	
			Stockasjön			
Rotatoria	3.8 ± 1.7	1–6	5	8 ± 1.6	6–10	4
Cladocera	2 ± 0.6	1–3		3 ± 1.2	2–5	
Total	7.4 ± 2.0	4–10		12 ± 3.7	8–17	
Volume	0.35 ± 0.30	0.14–0.87		1.42 ± 0.81	0.61–2.43	
			Skällingesjön			
Rotatoria	3.2 ± 1.2	2–5	7	4 ± 1	3–5	2
Cladocera	4 ± 1.4	2–6		3 ± 1	2–4	
Total	8.2 ± 1.2	7–10		8.5 ± 0.5	8–9	
Volume	0.96 ± 0.72	0.36–2.46		1.29 ± 0.24	1.05–1.52	
			Bossjön			
Rotatoria	3.5 ± 0.9	2–4	4	7 ± 2.4	3–10	5
Cladocera	3.8 ± 0.8	3–5		5.2 ± 1.2	2–7	
Total	8.5 ± 1.8	6–11		14 ± 2.8	10–17	
Volume	0.12 ± 0.07	0.07–0.22		1.58 ± 0.69	0.51–2.30	
			Stora Härsjön			
Rotatoria	3.5 ± 0.5	3–4	2	10.1 ± 2.1	5–12	8
Cladocera	4 ± 1	3–5		5.5 ± 1.8	3–8	
Total	8.5 ± 1.5	7–10		16.8 ± 1.4	15–20	
Volume	0.20 ± 0.22	0.29–0.14		0.77 ± 0.30	0.36–1.17	
			Tvärsjön			
Rotatoria	2.2 ± 1.9	0–7	6	6.2 ± 1.7	4–9	5
Cladocera	2.0 ± 0	2		3.8 ± 0.8	3–5	
Total	7.8 ± 2.7	3–11		12.0 ± 1.9	9–15	
Volume	0.55 ± 0.42	0.07–1.42		0.99 ± 0.39	0.65–1.59	

Year	Volume	Rotatoria	Cladocera plus Copepoda
		Stora Färgen	
1981	0.53	10	8
1982	1.01	8	9
1983	1.74	9	9
1984	0.58	8	10
1985	1.09	10	11
1986	1.23	9	9
1987	1.01	9	7
		Unacidified lakes (investigated in 1971 and 1976)	
$\bar{X}$	—	7.57	7
Range	—	5–11	4–11
SD	—	2	1.69

P content of the applied limestone powder, which was 0.03–0.04%. In Lake Gårdsjön, similar lime addition was calculated to increase the P concentration by about 3 μg/L, i.e., a much higher addition than that calculated to be added yearly from the surroundings (Broberg 1988).

In Lake Tvärsjön, the P concentration was on average significantly higher after the treatment, as in other lakes studied. The level changed more markedly in lakes with shorter water renewal times, as would be expected, since the water supply (including nutrients) is large compared with lake volume, and since precipitation mechanisms are less efficient in such lakes. The increased humic content, as a result of reduced sedimentation, also meant increased supply of several other compounds from the catchment area, since the humus acts as a chelator,

TABLE 7. Zooplankton composition (frequencies 1–5) in Lake Tvärsjön, July and August, 1974–87.

Taxon	Before liming								After liming					
	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987
Rotatoria														
Ascomorpha sp.		1					1	1						2
Asplanchna priodonta		2	4	4	1		4	3		4	3	1	2	2
Conochilus unicornis													4	
Collotheca sp.			3	2			2	2	2	1	2	3	2	
Gastropus stylifer													1	2
Kellicottia longispina					1		2			3		3	2	3
Keratella cochlearis	1		1							4	2	2	2	3
Keratella quadrata			1					1		1				
Keratella serrulata		1	1	2	1		2	1		1				
Lecane sp.										1				
Ploesoma hudsoni														2
Polyarthra remata	3	4	1	4	5		3	4		3	4	3	2	3
Polyarthra vulgaris			1				1				4	3	3	4
Synchaeta sp.													2	1
Cladocera														
Bosmina longispina	2	2	3	3	3	4	3	3	2	2	2	2	2	4
Ceriodaphnia quadrangula										2	3	2		
Daphnia cristata												2		3
Daphnia longispina												4	4	4
Diaphanosoma brachyurum											2	2	1	
Leptodora kindtii													1	
Holopedium gibberum	4	3	2	2	1	3	3	5		4	4	4		1
Polyphemus pediculus											1			
Sida crystallina											1			
Copepoda														
Cyclops spp.	1	1	1	1	1		1	1	4	2	4	4	4	4
Eudiaptomus gracilis	3		1	2	2	3	3	3	4	4	2	1	3	2

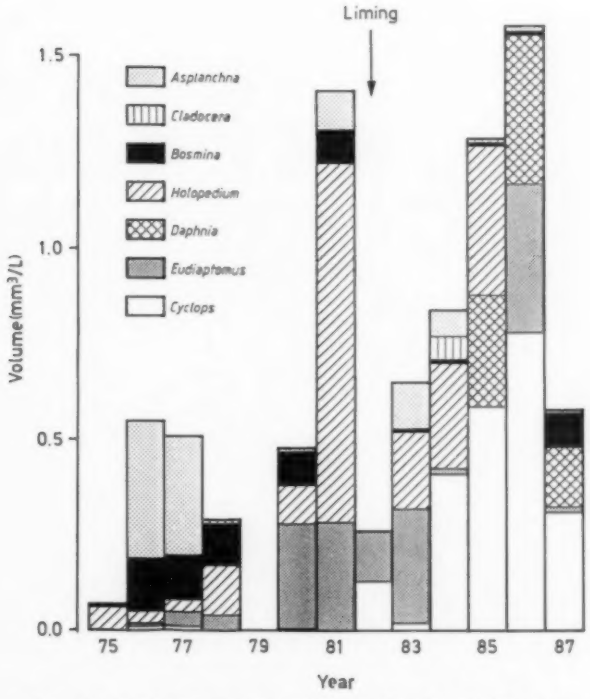

FIG. 10. Zooplankton volume and composition in Lake Tvärsjön.

keeping nutrients in suspension and accessible to the plankton. The surroundings of Lake Tvärsjön and others were not limed, and the supply of P from the catchment area probably remained low also after the liming. Thus, especially in view of the long water retention time, the increased P level was, with the exception of P addition by the limestone, probably related to mechanisms within the lake, in the first place probably to intensified microbial activity and increased decomposition of organic material (Gahnström 1988) and decreased adsorption of P to the sediments at high pH levels (Petterson and Olsson 1984).

Concerning the increased P levels, it is important to consider the special character of the studied lakes. The low runoff in combination with high Al concentrations at acidic conditions turns the lakes into traps for humus and P. This is in contrast with lakes with high runoff, where acidification changes the water quality little apart from the pH. This is exemplified by results from limed lakes of Central Sweden, where humus and P levels changed mainly in relation to climatic fluctuations (Hörnström et al. 1992).

Since the studied lakes are well oxygenated, and bacterial NO_3 reduction is restricted to anaerobic conditions, it is likely that the reduced level of NO_3-N in Lake Tvärsjön (Fig. 6) was an effect of raised primary production, especially since N deposition from air increased during the study period. The NO_3 reduction was, however, less marked in 1982 (the liming year), indicating that the lime P was less available to the primary producers. Reduced supply of other essential elements may, however, also be important. Thus, Bukaveckas (1989) suggested that the CO_2 depletion after liming reduces the production, since

TABLE 8. Zooplankton occurrence in six acidified lakes before and after liming compared with 10 unacidified lakes (Ref.) of the same region. T, Tvärsjön; St, Stockasjön; Sv, Stora Svansjön; Hä, Stora Härsjön; Bo, Bossjön; Sk, Skällingesjön. % = percentage of lakes where the species were recorded.

	Before liming													After liming												
	3–4 yr						1–2 yr							1–2 yr							3–4 yr						
Taxon	T	St	Sv	Bo	Sk	%	T	St	Sv	Hä	Bo	Sk	%	T	St	Sv	Hä	Bo	Sk	%	T	St	Sv	Hä	Bo	%	Ref. (%)
Ascomorpha sp.		+	+			40	+	+					33				+			17					+	+	40	80
Asplanchna priodonta	+	+		+		60	+	+			+	+	66	+	+	+	+	+		83	+	+	+	+	+	100	80
Conochilus unicornis		+		+		40		+		+	+		50		+	+	+			50	+	+		+	+	80	100
Collotheca sp.					+	20	+		+		+	+	66	+						17	+	+	+		+	80	10
Gastropus stylifer						0							0		+					17	+	+	+	+	+	100	10
Kellicottia longisp.	+		+	+	+	80	+			+		+	50	+			+	+	+	66	+	+	+	+	+	100	90
Keratella cochlearis		+		+	+	60		+			+	+	50	+	+	+	+	+	+	100	+	+	+	+	+	100	90
Keratella quadrata						0	+			+			33	+					+	33					+	20	0
Keratella serrulata	+	+			+	60	+	+			+	+	66	+						17						0	0
Ploesoma hudsoni						0							0		+	+	+	+		66		+	+	+		60	50
Ploesoma truncatum						0							0			+		+		33			+	+		40	10
Polyarthra remata	+	+	+	+	+	100	+	+	+	+	+	+	100	+	+		+	+	+	83	+	+		+	+	80	80
Polyarthra vulgaris			+			20	+		+	+			50	+	+	+	+	+	+	100	+	+	+	+	+	100	100
Synchaeta spp.						0							0		+	+	+	+		66	+	+	+	+		80	10
Trichocerca sp.						0							0							0						0	80
Bosmina longispina	+	+	+	+	+	100	+	+	+	+	+	+	100	+	+	+	+	+	+	100	+	+	+	+	+	100	100
Bythotrephes longimanus				+	+	40				+	+	+	50					+	+	33						0	10
Ceriodaphnia quadrangula						0							0	+			+			33	+	+	+	+	+	100	20
Daphnia cristata						0							0				+			17	+	+		+	+	80	80
Daphnia cucullata						0							0							0			+			20	10
Daphnia longispina						0							0				+			17	+			+		40	40
Diaphanosoma brachyurum				+	+	40				+	+	+	50	+			+	+	+	66	+		+	+	+	80	90
Holopedium gibberum	+	+				40	+	+		+	+		66	+	+		+	+		66	+	+	+	+	+	100	40
Leptodora kindtii						0				+			17				+	+	+	50	+			+	+	60	60
Polyphemus pediculus				+	+	40					+	+	33	+			+			33		+		+	+	60	20
Cyclops spp.	+	+		+		60	+	+			+		50	+			+	+	+	66	+	+		+	+	80	100
Eudiaptomus gracilis	+	+	+	+	+	100	+	+	+	+	+	+	100	+		+	+	+	+	83	+	+	+	+	+	100	90
Heterocope appendiculata						0							0							0						0	40
Chaoborus flavicans						0							0				+	+	+	50					+	20	20
Total no. of species	8	10	6	11	10		12	10	5	11	13	11		15	10	9	20	16	12		18	17	15	21	20		13–21

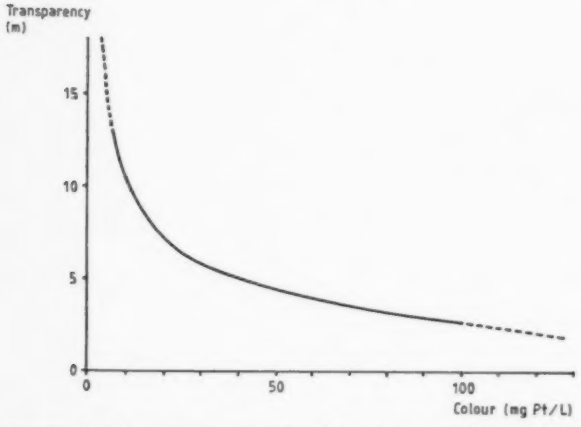

FIG. 11. Maximum transparency in relation to water colour in 1250 lakes (W. Dietrichson, Swedish Environmental Protection Agency, S 171 85 Solna, pers. comm.).

the plankton probably is still adapted only to CO_2 assimilation, i.e., the only C source occurring under acidic conditions.

The size distribution and species composition of the phytoplankton population must be considered when evaluating the production and trophic state of the lake. It is thus a well-known fact that growth rate and assimilation of algae are related to the surface/volume quotient of the cells (Malone 1980) and also to the algal group or species (Ramberg 1976). This is demonstrated by comparing the C assimilation per volume with the biomass composition (Ramberg 1976; Hörnström and Ekström 1986), indicating that the chlorophyceans are most efficient. The large algal volumes made up by dinoflagellates in Lake Tvärsjön in 1976 and 1978 (Fig. 8) were not the result of high production. Thus, an investigation in Swedish west coast lakes (in 1976), under minimum nutrient supply as a result of very low runoff in 1975–76 (Fig. 3) (Almer et al. 1978), showed that the largest phytoplankton biomasses occurred in the most acidic waters, i.e., those that had very low P levels.

After liming in Lake Tvärsjön during 1985–87 the algal volume again increased to around the average preliming level. The bulk of biomass, however, was mainly made up of chlorophyceans and chrysophyceans (Fig. 8), indicating increased epilimnic production, as in most other limed lakes. The lowered transparency of the water (Fig. 4), however, suggests that production per square metre may have been less affected.

The lime dosage and consequently the increased pH value seems to determine the development of the phytoplankton community. Thus, marked changes of the phytoplankton have not been recorded in limed lakes where pH did not exceed 6–6.5 (E. Hörnström, unpubl. data). However, in lakes where the alkalinity levels exceeded 0.20 meq/L, e.g., Lake Rishagerödvatten (Hörnström and Ekström 1986), Lake Hornasjön (Alenäs 1986), and Lake Gårdsjön (Larsson 1988), the availability of P increased markedly, resulting in water blooms and consequently high productivity.

The immigration rate of new phytoplankton taxa was species dependent. As in several other limed lakes (e.g., Larsson 1988), *Synedra nana*, as well as some chlorophyceans, occurred shortly after liming whereas other species did not become frequent until 3–4 yr after the treatment. This varying immigration rate might correspond to frequency and distribution of spores in the air. The varying growth rate of different species should also be important and should explain why chlorophyceans generally are dominants during the first year after liming.

The phytoplankton taxonomic composition is affected by factors such as pH and Al concentration (Hörnström et al. 1984) but is also governed by the level and quality of the nutrient supply (Hutchinson 1967; Rosén 1981), which in turn vary according to the specific soil and bedrock of the lake surroundings. Thus the phytoplankton of the limed lakes should not be expected to become similar to that in unacidified Lake Stora Färgen. However, comparison with unacidified lakes in the 1970's (Table 5) indicates that the species composition has not attained in all lakes a pattern that characterizes unacidified waters. Some species were rare or not recorded, while some that were atypical of oligotrophic lakes occurred. Thus, especially in 1986–87, the high frequency of, e.g., *Scenedesmus* spp. in Lake Tvärsjön indicated relatively eutrophic conditions, in spite of the low P level. In reference Lake Stora Färgen, all the cyanophyceans recorded in the older reference material were frequent during the whole period studied. Thus, it is not likely that the differences between the reference lakes and the limed lakes were the result of long-term trends changing the species composition also in unacidified lakes.

The species diversity of zooplankton in strongly acidic lakes is low (Brezonic et al. 1984). However, according to the observations in all our studied lakes, there is no clear relationship between species distribution and pH (Hörnström et al. 1984). Some researchers (e.g., Schaffner 1989) considered that *Daphnia* species are sensitive to low pH. However, Carter (1971) noted daphnids in acid ponds in Ontario, and Sarvala and Halsinaho (1990) found both *Daphnia cristata* and *D. longiremis* at pH 4.8 in Finnish lakes. The latter observations were, however, made only in humic waters, which indicates that the existence of humic compounds is essential for many zooplankters, especially at low pH.

The Al concentration remained around 300 μg/L before liming, and since humus with its ability to bind Al was completely absent, the tolerance level for some species, including that for *Daphnia* (Hörnström et al. 1984; Sarvala and Halsinaho 1990), was probably exceeded. Following liming, the Al concentration decreased to about 50 μg/L at the same time that the humus concentration gradually increased, suggesting that the Al toxicity was eliminated.

The total biomass of zooplankton, particularly within the Rotatoria and Cladocera, increased especially in Lake Bossjön, where the water colour increased markedly. There are several reasons for these changes. Humus substances provide a substrate for bacteria, the production of which in humic lakes may be considerable (Johansson 1983; Hessen et al. 1990). The bacteria are an important food source for the zooplankton (Nauwerck 1963; Pourriot 1977; Hakkari 1978), and despite the mostly oligohumic character of the studied lakes, the addition of humus was probably of major importance for the development of the zooplankton during 1984–87.

For various reasons, it may be difficult to find direct trophic relationships between phytoplankton and zooplankton (Frost 1980). Within the group Cladocera, which is the most investigated group in this respect on account of its large filtration capacity, some species apparently prefer oligotrophic conditions and thus low phytoplankton production, e.g., *Holopedium gibberum*, *Daphnia cristata*, and *Bythotrephes longimanus* (Pejler 1965; Hakkari 1978; Pejler 1983). Another major reason is that the food of filter-feeding species largely consists of detritus and bacteria. Hakkari (1978) thus reported that the proportion of such food among zooplankton in Finnish lakes was up to 90%, and similar results have been reported from Polish lakes (Gliwicz 1969) and a Swedish lake (Johansson et al. 1976). Under the assumption that zooplankton do not actively select food (Gliwicz 1969), the proportion of phytoplankton of suitable size should be determined by the availability of other organic material. In Lakes Tvärsjön and Skällingesjön, with low concentrations of humus, the increased phytoplankton diversity and production probably represented an important food resource and contribution to the development of the zooplankton.

During acidic conditions in Lake Tvärsjön the potential predators on zooplankton were few; waterbugs were at low density in the shoreline area, and fish were missing (unpubl. data). After liming, predators belonging to the bottom fauna were still negligible, but with the production of perch in 1983 and perhaps the increased abundance of Cyclops, the number of potential predators increased. However, this development took place in parallel with the increasing diversity and volume (not significant) of the zooplankton together with the increased occurrence of large cladocerans and copepods, and thus the occurrence of more suitable prey. The changed zooplankton composition after liming was therefore not the result of reduced predation pressure, but of improved water quality and increased food supply.

There is little information that could explain the zooplankton colonization of the limed lakes. However, both rotifers and some cladocerans have been reported to produce resting eggs that can persist in sediment for decades (Nipkow 1961; Moritz 1987; De Stasio Jr. 1990). After liming, when water quality is suitable, the eggs develop. Eggs may also be transported long distances by the wind or by aquatic birds to the limed lakes. The slow change in water quality, especially concerning the organic content, was however, probably a main hindrance for colonization during the first years after liming.

In summary, the liming led to markedly reduced Al levels and increased concentrations of P and humus, which increased the species richness and probably also the production of both phytoplankton and zooplankton. The comparison with the reference lakes shows that while the phytoplankton communities were not completely restored, a zooplankton community, similar to that in unacidified lakes, was developed. P levels were still low, but for natural reasons, i.e., geological characteristics of the area. The complementary fertilization that has been suggested should accompany liming (Blomqvist et al. 1989), adding a considerable cost, thus seems inappropriate, at least during the first years after liming.

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Improved Growth, Swimming Performance, and Muscular Development in Exercise-Conditioned Young-of-the-Year Striped Bass (*Morone saxatilis*)

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Exercise conditioning at 1.2–2.4 body lengths·s^{-1} for 60 d significantly improved final weights, specific growth rates (SGR), 2-min critical swimming velocities, red muscle cross-sectional areas at 80% standard length (SL), and red:white muscle ratio at 80% SL in both cultured and wild young-of-the-year striped bass (*Morone saxatilis*). Moreover, wild fish had final weights and SGR greater than cultured fish in both exercised and unexercised groups. Exercise conditioning also increased white muscle cross-sectional areas at all sections (50, 65, and 80% SL) in the cultured but not in the wild fish, although white muscle areas of exercised wild fish were significantly greater than in the unexercised cultured fish. It is suggested that exercise-conditioned striped bass used for stocking purposes might show increased survival rates in the wild.

Chez les jeunes bars rayés (*Morone saxatilis*) de l'année, d'élevage ou sauvages, l'exercice à raison de 1,2–2,4 longueurs corporelles·s^{-1} pendant 60 jours amène une amélioration statistiquement significative de divers paramètres : poids final, vitesse de croissance spécifique (VCS), vitesse de nage critique 2 min, section du muscle strié à 80 % de la longueur standard (LS) et rapport muscle strié/muscle lisse à 80 % de la LS. De plus, les poissons sauvages ont un poids final et une VCS supérieurs aux poissons d'élevage et ce, aussi bien dans le groupe soumis à l'exercice que dans le groupe non soumis à l'exercice. L'exercice a également augmenté la section des muscles lisses à 50, 65 et 80 % LS chez les poissons d'élevage, mais non chez les poissons sauvages, bien que la section des muscles lisses ait été statistiquement plus grande chez les poissons sauvages soumis à l'exercice que chez les poissons d'élevage non soumis à l'exercice. Il semblerait que les bars rayés destinés à régénérer les stocks pourraient avoir un meilleur taux de survis dans la nature s'il sont soumis à l'exercice.

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Since their introduction into California in 1879, striped bass (*Morone saxatilis*) have supported valuable fisheries in the Sacramento – San Joaquin estuary and adjacent coastal waters (Moyle 1976). Unfortunately, the striped bass populations have been declining steeply since 1974; in 1985 the adult population was just 25% of what it was in 1965 (Stevens et al. 1985). To minimize further reduction of the striped bass population, hatchery-reared young-of-the-year (YOY) and yearling striped bass, as well as wild fish salvaged from fish screens guarding water export pumps, have been stocked in the estuaries. Between 1981 and 1990, over 7 million striped bass were released (Villa 1991).

Exercise conditioning by sustained swimming for several weeks has been shown to provide many physiological benefits to growth, metabolic efficiency, muscular development, and swimming performance in various fishes (reviewed by Davison 1989). Wendt and Saunders (1973) have shown that forced exercise in Atlantic salmon (*Salmo salar*) smolts resulted in a greater percentage return as spawners; Creswell and Williams (1983) also showed higher recapture rates of exercised brown trout (*Salmo trutta*). Besner (1980) concluded that exercise training is an affordable rearing strategy to increase food conversion efficiency, stamina, growth, and survival of coho salmon (*Oncorhynchus kisutch*) used for mitigation purposes. If exercise conditioning similarly improves survival in striped bass, then such stocked bass would contribute more to the fishery, as well as increase the number of spawners to enhance natural reproduction. With this in mind, we investigated the effects of exercise conditioning in hatchery-reared (cultured) and wild YOY striped bass regarding two important factors in the survival of fish in the natural environment: growth and swimming performance.

The first objective was to test the hypothesis that exercise conditioning improves growth, swimming performance, and muscular development in cultured and wild YOY striped bass. The second objective was to determine the difference between cultured and wild YOY striped bass in their responses to exercise conditioning. To our knowledge, this was the first study to evaluate morphophysiological effects of exercise conditioning in a percichthyid fish.

Methods

Cultured YOY striped bass were obtained from the California Department of Fish and Game Central Valley Hatchery in Elk Grove. Wild YOY striped bass were salvaged from screen-

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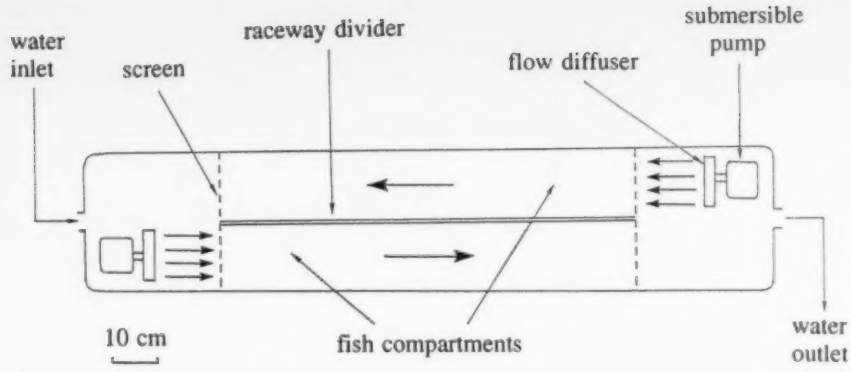

FIG. 1. Diagram of a raceway used for exercise conditioning (arrows indicate direction of water flow).

ing devices guarding water export pumps and held for 1 wk at the Department of Water Resources grow-out facility in Byron. Fish from both sources were quickly transported to the University of California, Davis, laboratory in insulated, fiberglass tanks with oxygenation levels near 100% air saturation and NaCl added to maintain salinity at 10 ppt to minimize stress-related mortality (Cech et al. 1990). Fish from each source were held separately in 1 × 2.3 m fiberglass tanks with a continuous flow of air-equilibrated well water and continuous aeration. Fish were held for 1 wk to allow them to recover from transport-related stress and fed with Silver Cup No. 2 trout pellets (Murray Elevators, Idaho).

After a 1-d fast, fish were individually weighed in a pre-tared beaker of water and randomly distributed among 12 plastic raceways. A total of 180 fish (each about 5 cm standard length (SL) weighing 3.3 ± 0.35 g) from each source was used. The stocking rate was 15 fish per replicate compartment with six replicates per source per exercise level. Each raceway was a black ABS plastic tank measuring 150 cm long, 30 cm wide, and 25 cm deep (Fig. 1). A fish screen was placed 30 cm from each end, and a PVC sheet divided the tank longitudinally to create two compartments. One compartment in each raceway was stocked with 15 cultured fish, while the other was stocked with 15 wild fish. A Teel submersible pump with an attached flow diffuser was placed beyond the screen at each end of the raceway to generate the desired water velocity in the exercise-conditioned treatments. Each raceway had a water depth of 15 cm with a continuous flow of air-equilibrated well water at 2 L·min^{-1} creating a flow of <1 cm·s^{-1}.

Half of the fish from each source were exercise conditioned at a water velocity of 6–12 cm·s^{-1} (equivalent to 1.2–2.4 body lengths (bl)·s^{-1}). Within compartments, the water velocity was about 12 cm·s^{-1} near the pump and about 6 cm·s^{-1} farthest from the pump. After 30 d, the water velocity was increased to 10–19 cm·s^{-1} by moving the pumps closer to the fish compartments. The increase in flow was necessary to correspond to the increase in fish size (mean SL = 8.5 cm) such that flow was equivalent to 1.2–2.2 bl·s^{-1}. The rest of the fish from both sources were reared in identical raceways (without pumps) with a water velocity of <1 cm·s^{-1} (<0.2 bl·s^{-1}). Water velocity was determined using a Marsh McBirney flowmeter.

Fish were fed Silver Cup No. 2 trout pellets at about 5% body weight four times daily for the first 2 wk, three times daily for the next 2 wk, and twice daily for the rest of the rearing period. Pumps were turned off during feeding and cleaning for about 40 min per feeding. Fecal matter and extra food were removed before the pumps were turned back on.

Temperature, dissolved oxygen content, and ammonia content of the water in each tank were monitored three times a week to ensure high water quality and minimal variability of these parameters in different tanks. Temperatures were read from minimum–maximum thermometers and diurnal variations were monitored using a Nester DO/temperature meter connected to a Linear chart recorder. Dissolved oxygen content was determined using a Nester DO/temperature meter and ammonia content was determined by the salicylate–hypochlorite spectrophotometric method (Bower and Holm-Hansen 1980). Fish were kept in a 12 h light : 12 h dark photoperiod regime.

At the end of the 60-d rearing period, fish were weighed and specific growth rates (SGR) were calculated using the following formula (Ricker 1979):

$$\mathrm{SGR} = 100 \cdot (\ln W_2 - \ln W_1) \cdot T^{-1}$$

where W_1 = weight of fish (grams) at the start of the experiment, W_2 = weight of fish (grams) at the end of the experiment, and T = duration of experiments (days).

Eighteen fish from each group per treatment (i.e., three fish from each compartment) were tested for swimming performance. Swimming performance was measured using a modified Brett-type swimming apparatus (Brett 1964) with a recirculating water flume incorporating a variable-speed motor. Each fish was placed in the swimming chamber and, after a 2-min rest, critical swimming velocity was measured by step increases of 10 cm·s^{-1} in water velocity at 2-min intervals until the fish was fatigued (following Beamish 1978). A fish was considered fatigued when it was "pinned" three times at the rear of the chamber. No correction factor for solid blocking effect was applied because the fish occupied <10% of the cross-sectional area of the swimming chamber (Bell and Terhune 1970). Critical swimming velocity (U_{crit}), defined as the maximum velocity that fish could maintain for a precise time period, was calculated using the following formula (Brett 1964):

$$U_{crit} = U_i + (U_{ii} \cdot T_i \cdot T_{ii}^{-1})$$

where U_i = highest velocity maintained for the prescribed time period (i.e., 2 min in our study), U_{ii} = velocity increment (i.e., 10 cm·s^{-1}), T_i = time elapsed (minutes) at the final velocity, and T_{ii} = prescribed swimming period (i.e., 2 min).

Immediately after the swimming performance test, each fish was quickly overanesthetized with MS-222 and frozen for mus-

TABLE 1. Mean (± SEM) initial and final weights (g), SGR (%·d^{-1}), 2-min critical swimming velocities (U_{crit}, cm·s^{-1}), red and white muscle cross-sectional areas (mm^2) at 50, 65, and 80%, and red:white muscle area ratios (R:W × 10) of unexercised and exercise-conditioned cultured and wild YOY striped bass. Values with different superscripts within a row are significantly different ($P < 0.05$).

	Cultured		Wild	
	Unexercised	Exercised	Unexercised	Exercised
Initial wt	3.32 (0.09)	3.30 (0.08)	3.27 (0.06)	3.23 (0.06)
Final wt	13.66 (0.34)^a	16.13 (0.36)^b	16.41 (0.36)^b	18.99 (0.38)^c
SGR	2.43 (0.03)^a	2.60 (0.02)^b	2.51 (0.03)^b	2.76 (0.04)^c
U_{crit}	58.17 (3.00)^a	76.50 (3.34)^b	60.43 (3.22)^a	74.02 (3.32)^b
Red				
50%	2.11 (0.23)	2.31 (0.27)	2.02 (0.21)	2.47 (0.35)
65%	1.68 (0.12)	2.38 (0.24)	2.07 (0.23)	2.33 (0.24)
80%	1.06 (0.07)^a	1.69 (0.16)^b	1.27 (0.10)^a	1.82 (0.16)^b
White				
50%	107.67 (3.92)^a	133.80 (6.85)^b	128.52 (3.99)^{ab}	142.95 (7.96)^b
65%	101.23 (3.14)^a	125.38 (5.53)^b	120.31 (4.24)^b	130.48 (5.42)^b
80%	50.32 (1.89)^a	60.10 (3.12)^b	55.49 (2.37)^{ab}	62.95 (2.84)^b
R:W				
50%	0.17 (0.02)	0.17 (0.02)	0.16 (0.02)	0.17 (0.02)
65%	0.17 (0.01)	0.18 (0.02)	0.17 (0.02)	0.18 (0.02)
80%	0.21 (0.01)^a	0.28 (0.02)^b	0.23 (0.02)^a	0.29 (0.02)^b

cle area measurements. Before each measurement, the fish was partially thawed and cross-sectioned at 50, 65, and 80% SL and slide photographs of the sections were taken. Slides were projected onto a digitizing pad for tracing and red and white muscle areas were calculated using the Microplan II imaging program.

Analysis of variance (ANOVA) and Tukey's test were used to find significant differences in initial and final weights, SGR, critical swimming velocities, and the cross-sectional areas of red and white muscle. Kruskal–Wallis ANOVA was used to determine significant differences in the red:white muscle ratio. All statistical analyses were conducted using SYSTAT software.

Results

Temperature, dissolved oxygen content, and ammonia content of the water did not vary among raceways. Temperature in the raceways fluctuated from a minimum of 18.3°C (±0.07) at night to a maximum of 24.9°C (±0.06) during the day with a mean temperature of 21.7°C (±0.57). Dissolved oxygen content remained high at 7.6 mg·L^{-1} (±0.03) and ammonia content was always low at 0.13 ppm (±0.01) throughout the 60-d rearing period. No mortality occurred in the 60-d rearing period.

Exercise conditioning at 1.2–2.4 bl·s^{-1} for 60 d significantly improved growth in both cultured and wild YOY striped bass. Initial weights in all groups were not statistically different, but final weights in the exercise-conditioned fish were greater than in unexercised ones (Table 1) in both cultured and wild groups (18 and 16% higher, respectively). Wild YOY had final weights greater than cultured fish in both exercised and unexercised groups. SGR for exercised wild fish was also higher than that of both unexercised and exercised cultured fish (14 and 6% higher, respectively) as well as higher than that of unexercised wild fish (10% higher).

Exercise conditioning also significantly improved the swimming performance of YOY striped bass. Exercised fish had a greater mean 2-min critical swimming velocity than the unexercised ones in both the cultured and wild groups (32 and 19% higher, respectively) (Table 1).

Mean cross-sectional areas of red muscle at 50 and 65% SL in exercised cultured fish were not statistically greater than those in unexercised fish from the same source (Table 1). At 80% SL, red muscle cross-sectional areas of exercised cultured and wild fish were significantly greater than those of unexercised fish.

Cross-sectional areas of white muscle in the exercised cultured fish were greater than those in unexercised fish at all sections (Table 1). In the wild fish, however, white muscle increases due to exercise conditioning were not significant, although unexercised wild fish had white muscle areas at 65% SL significantly greater than those in unexercised cultured fish.

The red:white muscle area ratios in exercised cultured and wild fish were significantly greater than in the unexercised cultured fish only at 80% SL (Table 1).

Discussion

Exercise conditioning at 1.2–2.4 bl·s^{-1} for 60 d enhanced growth in YOY striped bass. Similar observations were also shown in coho salmon (Besner 1980), rainbow trout (*Oncorhynchus mykiss*; Nahhas et al. 1982a; Houlihan and Laurent 1987; Farrell et al. 1990), brook trout (*Salvelinus fontinalis*; Leon 1986; East and Magnan 1987), Atlantic salmon (*Salmo salar*; Totland et al. 1987), and Arctic char (*Salvelinus alpinus*; Christiansen et al. 1989; Christiansen and Jobling 1990). The growth increases in exercised fish could be the results of factors such as (1) better food conversion efficiency (Davison and Goldspink 1977), (2) increased growth hormone levels (Barrett and McKeown 1988a, 1988b), (3) increased protein synthesis rates (Houlihan and Laurent 1987; Christiansen et al. 1989), (4) reduced antagonistic behavior (East and Mangan 1987; Christiansen et al. 1989; Christiansen and Jobling 1990), and (5) reduced stress levels (Woodward and Smith 1985).

There are several possible reasons why our wild YOY striped bass, both exercised and unexercised, grew significantly faster than our cultured striped bass. Natural selection pressures (e.g., predation and disease) and the salvage operation may have eliminated less-fit individuals from the group of fish salvaged

from the pump screens. The salvage operation involved diversion of fish from the main current, concentration in a collecting tank, capture, crowding in a lift bucket, and transfer into a transport truck (Skinner 1974). Also, the wild fish probably benefitted from natural exercise conditioning through exposure to strong water currents, and some effects of such conditioning possibly remained at the end of the 60-d rearing period.

Many studies on fish exercise conditioning have focused on changes in swimming performance. Our results showed that exercise conditioning improves the swimming performance of YOY striped bass. Similar results were shown in largemouth bass (*Micropterus salmoides*; Farlinger and Beamish 1978), roach (*Rutilus rutilus*; Broughton et al. 1980), coho salmon (Besner and Smith 1983), brook trout (Leon 1986), and rainbow trout (Nahhas et al. 1982b; Houlihan and Laurent 1987; Pearson et al. 1990). Improved swimming performance is believed to be related not only to an increase in muscle tissues, but also to a lower oxygen consumption rate at any given swimming speed (Nahhas et al. 1982b; Woodward and Smith 1985). The aerobic capacity of red and white muscle has been shown to increase in rainbow trout (Davie et al. 1986) and Atlantic salmon (Totland et al. 1987) with exercise conditioning. Farlinger and Beamish (1987) found increased hemoglobin in exercised largemouth bass, indicating an enhancement of aerobic capacity.

Muscle is the active tissue involved in exercise training. In our study, exercise conditioning through sustained swimming increased the red muscle tissue at 80% SL in cultured and wild fish. Broughton et al. (1980) also found a greater increase in red muscle mass in the more posterior muscle in roach exercised at 2.6–3.3 $bl \cdot s^{-1}$ for 30–40 d. This indicates that the red muscles in this region are principally involved in maintaining the fish in the water flow. Greer Walker and Pull (1973) considered that the muscles in this region underwent the greatest contraction during swimming. Davie et al. (1986) also showed an increasing trend in red muscle enlargement towards the caudal portion of the body (60–80% fork length) in rainbow trout exercised at 1 $bl \cdot s^{-1}$ for 200 d.

Exercise conditioning also resulted in an increase in white muscle in all sections in our cultured striped bass. Many previous studies on several species have reported increased white muscle size following exercise training (Davison and Goldspink 1977, 1978; Greer Walker and Emerson 1978; Johnston and Moon 1980; Totland et al. 1987). Johnston and Moon (1980) showed that the threshold swimming speed for white muscle recruitment in brook trout is about 1.8 $bl \cdot s^{-1}$, indicating that muscular development (as cross-sectional area) may be related to recruitment of white fibers at exercise conditioning velocities.

It is interesting to note that increases in white muscle in the exercised wild fish were not significantly different from those in the unexercised wild fish but were significantly greater than from those in the unexercised cultured fish. Furthermore, the white muscle area at 65% SL in unexercised wild fish was significantly greater than that in the unexercised cultured fish. This could be due to effects of natural "exercise conditioning" in the unexercised wild fish remaining even after 60 d, such that their increases in white muscle were comparable with those in the exercised fish.

The ratios of red:white muscle area at 80% SL in both exercised cultured and exercised wild fish were greater than those in the unexercised fish, indicating mostly aerobic muscular benefits of conditioning at speeds of 1.2–2.4 $bl \cdot s^{-1}$ in YOY striped bass. Nahhas et al. (1982b) demonstrated that low-speed (1.4 $bl \cdot s^{-1}$) training for 46 d in small rainbow trout did not alter the ratio of red:white muscle, but at medium to high speeds (2.2 and 3.5 $bl \cdot s^{-1}$), the proportion of white muscle increased. Interestingly, studies on roach (Broughton et al. 1980) and brook trout (Johnston and Moon 1980) showed increases in *red* muscle at high speeds (2.6–3.3 and 3 $bl \cdot s^{-1}$, respectively).

Faster growth and enhanced swimming performance should directly affect the survival of YOY striped bass stocked in aquatic habitats. Survival of stocked fish has been shown to increase when they are stocked at larger sizes. Higher growth rates shorten the period of time spent as potential prey and also increase prey-capture efficiency as predators (Junge and Phinney 1963; Townsend and Calow 1981). Better swimming performance improves feeding efficiency and enhances both the escape response from predators and the capacity to maintain position against a current (Beamish 1978). This is especialy important for YOY striped bass stocked into the Sacramento – San Joaquin delta system where the current can increase suddenly, particularly near the water export pumps (Skinner 1974). Besner and Smith (1983) concluded that long-term conditioning regimes at low water velocities for coho salmon prior to release into the natural environment result in a more efficient use of energy during their oceanic migrations.

In summary, we have shown for the first time, that a percichthyid fish shows increased growth, improved swimming performance, and increased muscular development in response to exercise conditioning in the laboratory. If YOY striped bass used for fisheries enhancement are exercise conditioned before their release, then return and recapture percentages may be enhanced, as shown in Atlantic salmon (Wendt and Saunders 1973) and brown trout (Creswell and Williams 1983), thereby significantly increasing the spawning striped bass population.

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Mitochondrial DNA Variation in Chinook (*Oncorhynchus tshawytscha*) and Chum Salmon (*O. keta*) Detected by Restriction Enzyme Analysis of Polymerase Chain Reaction (PCR) Products

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We analyzed intraspecific mitochondrial DNA variation in chinook salmon (*Oncorhynchus tshawytscha*) from drainages in the Yukon River (Alaska and Yukon Territory), the Kenai River (Alaska), and Oregon and California rivers; and chum salmon (*O. keta*) from the Yukon River and Vancouver Island, and Washington rivers. For each species, three different portions of the mtDNA molecule were amplified separately using the polymerase chain reaction and then digested with at least 19 restriction enzymes. Intraspecific sequence divergences between haplotypes were less than 0.01 base substitution per nucleotide. Nine chum salmon haplotypes were identified. Yukon River chum salmon stocks displayed more haplotypes (eight) than the stocks of Vancouver Island and Washington (two). The most common chum salmon haplotype occurred in all areas. Seven chinook salmon haplotypes were identified. Four haplotypes occurred in the Yukon and Kenai rivers and four occurred in Oregon/California, with only one haplotype shared between the regions. Sample sizes were too small to quantify the degree of stock separation among drainages, but the patterns of variation that we observed suggest utility of the technique in genetic stock identification.

Nous avons analysé les variations de l'ADN mitochondrial intraspécifique chez le saumon chinook (*Oncorhynchus tshawytscha*) dans les bassins hydrographiques du fleuve Yukon (Alaska et territoire du Yukon), de la rivière Kenai (Alaska) et des rivières de l'Oregon et de la Californie, ainsi que chez le saumon kéta (*O. keta*) du fleuve Yukon et de rivières dans l'île Victoria et dans l'État de Washington. Dans la cas de chacune des espèces, trois segments différents d'ADNmt ont été amplifiés séparément par réaction en chaîne à la polymérase, puis digérés par au moins 19 enzymes de restriction. Les divergences dans les séquences intraspécifiques entre les haplotypes étaient inférieures à 0,01 substitution de bases par nucléotide. Neuf haplotypes de saumon kéta ont été reconnus. Les stocks de saumons kéta du fleuve Yukon ont montré plus d'haplotypes (huit) que les stocks de l'île Victoria et de l'État de Washington (deux). L'haplotype de saumon kéta le plus fréquent a été observé dans toutes les régions étudiées. Sept haplotypes de saumon chinook ont été reconnus. Quatre haplotypes ont été observés dans le fleuve Yukon et la rivière Kenai et quatre dans les rivières de l'Oregon et de la Californie, un seul haplotype étant commun aux différentes régions étudiées. La taille des échantillons était trop faible pour que l'on puisse quantifier le degré de séparation des stocks entre les bassins hydrographiques, mais la répartition de la variation que nous avons observée laisse entrevoir l'utilité de la technique pour l'identification génétique des stocks.

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Chinook salmon (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) are two commercially important species that spawn in Alaska, western Canada, Washington, Oregon, and northern California. Informed management of salmon fisheries requires the ability to identify different breeding groups (stocks) for determining escapement, harvest allocation, impact assessment, and transplant strategy. For example, an unknown proportion of the breeding stock of chinook and chum salmon of the Yukon River system is harvested in the Pacific Ocean and Bering Sea before the fish enter the river enroute to spawning grounds. Once the fish enter the river, over 90% of the harvest is by U.S. fishermen. Assessment of the impact of the ocean fishery and allocation of the harvest in

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TABLE 1. Distribution of adult chum salmon mtDNA haplotypes among sample locations. Chulinak, Chandalar, Sheenjek, Fishing Branch, and Minto are Yukon River locations. AK, Alaska; YT, Yukon Territory.

Sample location	Year	Haplotype									Total
		C1	C2	C3	C4	C5	C6	C7	C8	C9	
Chulinak, AK	1989	8	—	—	—	—	—	—	—	1	9
Chandalar, AK	1989	1	—	4	1	—	—	—	1	—	7
Sheenjek, AK	1989	3	—	3	—	2	1	—	1	—	10
Fishing Branch, YT	1989	2	—	1	—	—	2	—	—	—	5
Minto, YT	1989	4	—	4	—	—	1	1	—	—	10
Vancouver Island	1990	4	—	—	—	—	—	—	—	—	4
Washington	1990	4	1	—	—	—	—	—	—	—	5

the river among U.S. and Canadian fishermen are important issues.

Fish stocks can be identified on different geographic scales. For example, high-seas fisheries may harvest salmon destined to breed in Asia or North America. On a smaller scale, drainages within a river system may contain partially or completely isolated stocks. The relative contribution of each source in the harvest can be estimated only if stocks can be identified.

Genetic variation has been used extensively in efforts to discriminate fish stocks. Protein electrophoresis is presently the most widespread technique used for genetic stock identification. An advantage of protein electrophoresis for stock identification is the ability to detect variation at many loci for many fish. Disadvantages include the need for high-quality, undegraded tissues and the relatively low resolution of genetic variation. Protein electrophoresis data have been used to differentiate U.S. and Canadian chum and chinook salmon stocks in the Yukon River (Wilmot et al. 1992).

Analysis of the DNA molecule can provide better resolution of genetic variation than does protein electrophoresis, but the number of loci that can be analyzed in salmon is currently limited. In addition, DNA laboratory procedures are generally more time consuming and expensive than protein electrophoresis procedures. Most analyses of DNA variation in natural populations have focused on mitochondrial DNA (mtDNA) because it can be isolated from the nuclear DNA and often shows intraspecific variation. In most vertebrates studied, mtDNA is maternally inherited without recombination and its rate of evolution and differentiation among populations is thought to be approximately four times faster than that of nuclear genes (Birky et al. 1983; A.C. Wilson et al. 1985). Thus, it has been predicted that populations will show spatial or temporal subdivision of mtDNA genotypes which is not apparent for the nuclear genome.

mtDNA variation has been examined among stocks of several species of fish including salmon (Gyllensten and Wilson 1987; Grewe and Hebert 1988; Davidson et al. 1989; Carr and Marshall 1991). Anadromous and nonanadromous Atlantic salmon (*Salmo salar*) stocks were differentiated by restriction enzyme analyses (Palva et al. 1989; Birt et al. 1991). G.M. Wilson et al. (1985) found mtDNA differentiation among geographic areas in rainbow trout (*O. mykiss*), and Grewe and Hebert (1988) reported considerable geographic structure among stocks of lake trout (*Salvelinus namaycush*) in the Great Lakes. Chum salmon from two locations in British Columbia differed by a single restriction site which suggested a degree of geographic structuring (Thomas et al. 1986). Wilson et al. (1987) reported geographic structure of mtDNA variation among chinook salmon from Alaska and British Columbia. Interspecific mtDNA variation in salmon has also been studied in some detail (Berg and Ferris 1984; Thomas et al. 1986; Thomas and Beckenbach 1989).

The recent development of the polymerase chain reaction (PCR; Saiki et al. 1988) has resulted in more efficient analyses of mtDNA variation in natural populations (Kocher et al. 1989), including fish stocks (Carr and Marshall 1991). With PCR, specific sequences in the DNA molecule (e.g., mtDNA) are selectively amplified using sequence-specific oligonucleotide primers. The technique allows much faster analyses than are permitted by traditional DNA methods and has the capability of using somewhat degraded tissues. PCR-amplified DNA segments can be analyzed by direct sequence analysis (e.g., Carr and Marshall 1991) or restriction enzyme analysis. Restriction enzyme analysis of PCR products has been done on other species (Pinder et al. 1991; Karl et al. 1992) and we report here extension of the methods to mtDNA of salmon.

We have two objectives in this paper: to describe PCR methods for analysis of salmon mtDNA and to present a preliminary assessment of mtDNA variation of chinook and chum salmon on different geographic scales. We describe mtDNA variation in chinook salmon from the Yukon and Kenai rivers and the Pacific Northwest (i.e., Oregon and California) and in chum salmon from the Yukon River and the Pacific Northwest (i.e., Vancouver Island and Washington).

Materials and Methods

Skeletal muscle, liver, or heart samples were collected from several locations and stored at −70°C until processed (Tables 1 and 2; Fig. 1). Genomic DNA from Vancouver Island and Washington chum salmon was extracted from about 50 mg of tissue (powdered with liquid nitrogen) incubated in 500 μL of STE buffer (0.1 M NaCl, 10 mM Tris–HCl, 1 mM EDTA, pH 8.0), 50 μL of 20% SDS (sodium dodecyl sulfate), and 10 μL of proteinase-K (10 mg·mL^{-1}) at 65°C for at least 30 min. Ammonium acetate (250 μL of a 4°C, 7.5 M stock) was then added, the samples incubated on ice for 1 h, centrifuged at 9000 × *g* for 5–10 min, and the supernatant transferred to new tubes. Nucleic acids were precipitated with 1 mL of 95% ethanol and centrifuged at 9000 × *g* for 5–10 min; the 95% ethanol was decanted, and the nucleic acid pellet was washed with 70% ethanol. Nucleic acid pellets were air-dried and resuspended in deionized water or TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6).

For all other tissue samples, we extracted genomic DNA from about 0.5 g of tissue by incubation in 1 mL of extraction buffer (0.2 M Tris–HCl, 0.1 M EDTA, pH 8.5), 10% SDS, in the presence of 0.25 μg proteinase-K at 60°C for 2 h. In some

TABLE 2. Distribution of chinook salmon mtDNA haplotypes among sample locations. Andreafsky, Anvik, Pelly, and Takhini are Yukon River locations. AK, Alaska; YT, Yukon Territory; OR, Oregon; J, juvenile; A, adult.

Sample location	Year	Maturity	Haplotype							Total
			K1	K2	K3	K4	K5	K6	K7	
Kenai, AK	1988	J	7	4	1	—	—	—	—	12
Andreafsky, AK	1988	A	4	5	—	—	—	—	—	9
Anvik, AK	1988	A	5	3	—	—	—	—	—	8
Pelly, YT	1989	J	1	2	—	—	—	—	—	3
Takhini, YT	1990	J	7	2	—	1	—	—	—	10
Willamette, OR	1990	A	—	1	—	—	3	—	1	5
Scott, CA	1990	J	—	—	—	—	1	4	—	5
Yuba, CA	1990	J	—	—	—	—	5	—	—	5

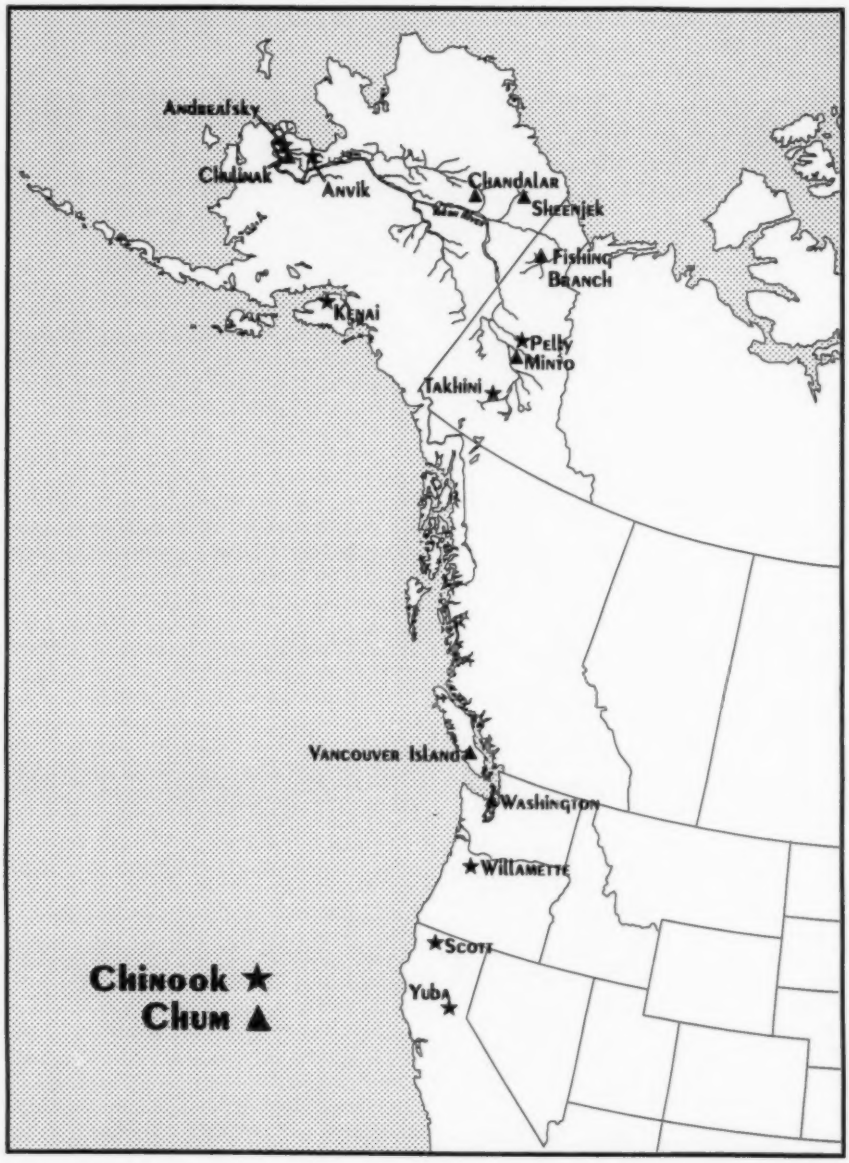

FIG. 1. Map showing the sample locations of chum and chinook salmon.

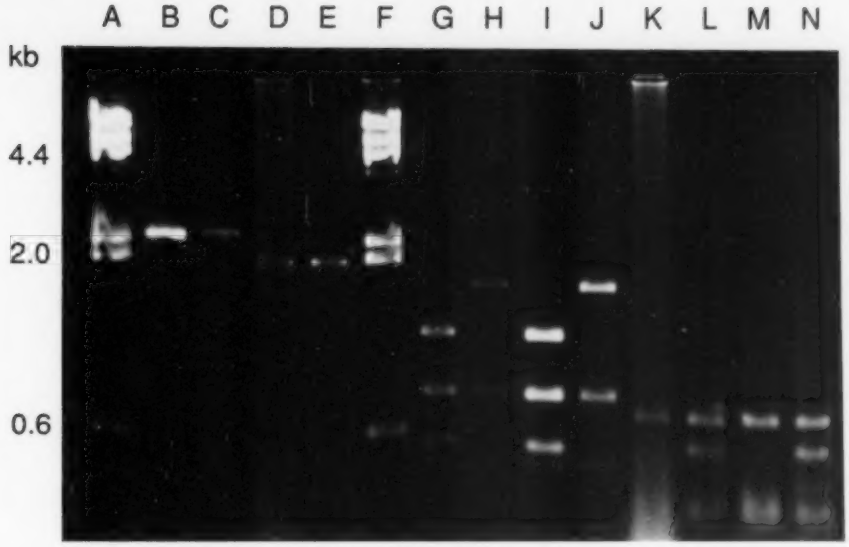

FIG. 2. Photograph of a gel showing band patterns of PCR products and restriction digest products in chinook salmon. Lanes A and F are lambda phage cut with *Hin*d III. Uncut mtDNA segments are in lanes B and C (ND-5/6) and lanes D and E (ND-1). Fragment patterns for ND-5/6 digested with *Bst*U I are in lanes G–J, showing two variants (lanes G and I versus H and J). Fragment patterns for ND-1 digested with *Rsa* I are in lanes K–N, showing two variants (lanes K and M versus L and N; kb = kilobase).

cases, samples were left overnight at 37°C. Potassium acetate (650 μL of a 5 M stock) was then added and the solution chilled on ice for 30 min, centrifuged at 9000 × *g* for 30 min (Davis et al. 1980), and the supernatant extracted once with a phenol – chloroform – isoamyl alcohol mix (25:24:1) and once with chloroform–isoamyl alcohol (24:1). Nucleic acids were precipitated with an equal volume of isopropanol and centrifuged at 9000 × *g* for 30 min. The isopropanol was then decanted, and the nucleic acid pellet was washed in ethanol, air-dried, and then resuspended in 200 μL of TE buffer.

Four mtDNA segments were amplified with PCR using the following primers:

NADH dehydrogenase-1 (ND-1)
5′-ACCCCGCCTGTTTACCAAAAACAT-3′
5′-GGTATGAGCCCGATAGCTTA-3′

ND-5/6
5′-AATAGTTTATCC(A/G)TTGGTCTTAGG-3′
5′-TTACAACGATGGTTTTTCAT(G/A)TCA-3′

ND-3/4
5′-TAA(C/T)TAGTACAG(C/T)TGACTTCCAA-3′
5′-TTTTGGTTCCTAAGACCAA(C/T)GGAT-3′

D-loop
5′-TTGGGTTTCTCGTATGACCG-3′
5′-AGAGCGTCGGTCTTGTAAACC-3′

These primers were developed by comparing the mtDNA sequences of cow (*Bos*; Anderson et al. 1982), human (*Homo*; Anderson et al. 1981), frog (*Xenopus*; Roe et al. 1985), and mouse (*Mus*; Bibb et al. 1981). Primers were located in the conserved tRNA and rRNA sequences which flank the amplified fragment. Descriptions of the exact locations of these primer sequences in the mtDNA molecule are available from the authors. The locations of the mtDNA segments (ND-1, ND-3/4, ND-5/6, and D-loop) are described in Anderson et al. (1982).

MtDNA segments ND-1, ND-5/6, and D-loop were analyzed in chinook salmon, and mtDNA segments ND-3/4 and ND-5/6 were analyzed in chum salmon. ND-1 was analyzed in some chum salmon, but some samples did not amplify, so this segment was not used for defining haplotypes.

Each PCR reaction was composed of 0.1–0.5 μg of genomic DNA, 5 μL of 10× buffer (0.1 M Tris–HCl, pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl, 1 $\mu g \cdot \mu L^{-1}$ bovine serum albumin), 5 μL of dNTP mix (2 mM each of dATP, dTTP, dCTP, and dGTP in 10 mM Tris–HCl, pH 7.9), 5 μL of a 2 μM solution of each of two primers, 1.25–2.50 units of *Taq* polymerase, and deionized water added for a final volume of 50 μL. The amplification cycle consisted of 95°C for 45 s, 50°C for 30 s, and 70°C for 2 min and 30 s. Reactions were cycled 32 times. Amplified segments were digested with at least 19 restriction enzymes (Appendix 1), run on 2% agarose gels with TBE or TAE buffer (Sambrook et al. 1989), stained with ethidium bromide, and photographed with polaroid film under ultraviolet light (wavelength = 312 nm).

The sizes of restriction fragments were estimated by comparison with a standard (lambda phage DNA digested with *Hin*d III or *Bst*E II). Restriction fragment patterns were visually identified from the gels and photos. Composite haplotypes were defined from the restriction fragment patterns for all mtDNA segment – restriction enzyme combinations (Lansman et al. 1981). We calculated the proportion of restriction fragments shared among haplotypes (F) and nucleotide sequence divergence (p, base substitutions per nucleotide) with the fragment method of Upholt (1977). For each haplotype, we combined fragments for all the amplified mtDNA segments (e.g., ND-1, ND-5/6, and D-loop for chinook salmon). We calculated F and

TABLE 3. Composite mtDNA haplotype definitions for chum salmon. Letters refer to restriction fragment patterns (Appendix 1) that occur in individual salmon.

mtDNA segment	Restriction enzyme	Haplotype definition								
		C1	C2	C3	C4	C5	C6	C7	C8	C9
ND-5/6	*Alu* I	A	A	A	A	C	A	A	A	A
	Ase I	B	A	B	B	B	B	C	B	B
	Dpn II	B	B	B	B	B	B	B	C	B
	Rsa I	B	B	C	C	B	B	B	B	B
	*Sau*96 I	B	B	B	B	B	B	B	C	D
	*Bsa*J I	B	B	B	B	B	B	C	B	B
	Taq I	B	B	B	B	C	C	B	B	B
ND-3/4	*Rsa* I	B	B	B	B	B	B	C	B	B
	*Bst*U I	B	B	B	C	B	B	B	B	B

TABLE 4. Composite mtDNA haplotype definitions for chinook salmon. Letters refer to restriction fragment patterns (Appendix 1) that occur in individual salmon.

mtDNA segment	Restriction enzyme	Haplotype definition						
		K1	K2	K3	K4	K5	K6	K7
ND-5/6	*Bst*U I	A	C	C	C	A	A	A
ND-1	*Ase* I	A	A	A	A	A	C	A
	Hae III	A	A	A	A	A	A	C
	Rsa I	A	C	A	C	C	C	C
	Ava II	A	A	A	A	A	A	C
D-loop	*Bsa*J I	A	A	A	D	C	C	C
	*Bst*N I	A	A	A	A	C	C	C

p separately for four-, five-, and six-base enzymes. A final p was calculated as an average, weighted by the number of restriction fragments for each type of enzyme (four, five, or six bases; Kessler and Avise 1985). Our estimates of p should be considered rough approximations of mtDNA divergences because our restriction fragments were generated from several separate linear fragments from different parts of the mtDNA molecule, we did not map restriction sites, and we could not resolve fragments smaller than about 300 base pairs on the gels.

Results and Discussion

The sizes of PCR-amplified mtDNA segments for chum salmon were as follows: ND-1, 2.0 kilobases (kb); ND-5/6, 2.4 kb; and ND-3/4, 2.3 kb. For chinook salmon the sizes were as follows: ND-1, 2.0 kb; ND-5/6, 2.4 kb; and D-loop, 1.3 kb. Typical amplified segments and restriction fragment patterns are shown in Fig. 2. Restriction fragment patterns that varied within species are described in Appendix 2 and composite haplotypes are defined in Tables 3 and 4.

Chum Salmon

Restriction site variation was detected for ND-3/4 (*Rsa* I, *Bst*U I), ND-5/6 (*Alu* I, *Ase* I, *Dpn* II, *Rsa* I, *Sau*96 I, *Bsa*J I, *Taq* I; Appendix 2), and ND-1 (*Dpn* II, *Bst*N I). Because some samples did not amplify for ND-1, this segment was not used for haplotype definitions or sequence divergence estimates in chum salmon. Nine mtDNA haplotypes were identified in chum salmon (Table 3). Sequence divergences between the haplotypes were low, ranging from $p = 0.00060$ ($F = 0.99$; haplotypes C1 and C6, and C3 and C4) to $p = 0.0062$ ($F = 0.92$; haplotypes C5 and C7). Thomas et al. (1986) also reported low mtDNA divergence ($p = 0.0024$) for chum salmon in British Columbia. Low mtDNA differentiation of chum salmon populations was also indicated by the occurrence of the most common haplotype, C1, in both the Yukon River and Pacific Northwest. Thus, sequence divergence and haplotype distribution indicated recent common mtDNA ancestry of the sampled populations.

Different haplotypes occurred in the different Yukon River drainages, but sample sizes were too small to quantify differentiation. The five Yukon River locations represent one summer-run stock (Chulinak) and four fall-run stocks (Chandalar, Sheenjek, Minto, and Fishing Branch). The summer run generally occurs from early June through mid-July. The fall run overlaps with the summer run, continuing through September (Buklis 1981). Morphological differences between summer- and fall-run stocks were described by Bergstrom et al. (1991). Analysis of 19 protein loci with electrophoresis showed that Yukon River summer- and fall-run stocks had well-differentiated allele frequencies (Wilmot et al. 1992). The summer-run stock had a relatively high frequency of mtDNA haplotype C1. One summer-run fish had haplotype C9 which was not observed in the fall-run stocks. The number of different haplotypes for any one fall-run stock ranged from three to five, while there were two haplotypes in the summer-run stock (Table 1). Larger sample sizes are needed to quantify mtDNA differentiation for stock identification programs.

Chinook Salmon

Restriction site variation was detected in ND-1 (*Ase* I, *Hae* III, *Rsa* I, *Ava* II), ND-5/6 (*Bst*U I), and D-loop (*Bsa*J I,

*Bst*N I). Seven mtDNA haplotypes were identified in chinook salmon (Table 4). Like chum salmon, sequence divergences were low, ranging from $p = 0.00030$ ($F = 0.99$; haplotypes K2 and K4) to $p = 0.0044$ ($F = 0.94$; haplotypes K3 and K7). Wilson et al. (1987) also reported intraspecific mtDNA sequence divergences of less than $p = 0.01$ in chinook salmon from Alaska and British Columbia.

Different haplotypes occurred in two geographic regions, the Yukon and Kenai rivers and the Pacific Northwest (Table 2). Only haplotype K2 occurred in both regions. Analysis of 22 protein loci with electrophoresis has shown lower Yukon River stocks (Andreafsky and Anvik) and upper Yukon River stocks (Takhini and Pelly) to have well-differentiated allele frequencies (Wilmot et al. 1992). MtDNA haplotype distribution within the Yukon River did not show obvious frequency differences between sample locations (Table 2). As with chum salmon, larger sample sizes are needed to quantify mtDNA differentiation for stock identification.

Evolutionary Genetics and Gene Flow

It is important to distinguish between two forms of mtDNA differentiation: nucleotide sequence divergence of haplotypes versus the number of different haplotypes and their spatial distribution. Nucleotide sequence divergence can be useful in describing phylogenetic relationships of haplotypes, but not necessarily relationships of populations or species (Pamilo and Nei 1988). The number of haplotypes and their spatial distribution may be more useful for assessment of structure and gene flow among populations (Avise et al. 1990; Cronin 1991). This is because gene flow and colonization from different source populations may result in the mixing of haplotypes with different sequence divergences (Slatkin 1989; Wayne et al. 1990; Cronin et al. 1991).

mtDNA sequence divergences were generally low for chinook and chum salmon and haplotypes were shared among areas. This pattern may occur in areas that have recently been colonized following glaciation (Slatkin 1987; Avise 1989; Cronin 1992). Bartley and Gall (1990) suggested that chinook salmon recently colonized glaciated areas of Alaska and Canada from the Columbia River and Bering Sea beginning about 10 000 yr ago.

Colonization of vacant habitat may result in a founder effect (Mayr 1963) of reduced number of haplotypes. The numbers and distributions of mtDNA haplotypes for both species do not suggest that there is reduced variation in Alaska and do not necessarily support radiation from a southern refugium. It is possible that in addition to a Pacific Northwest glacial refugium, there was a central Alaskan refugium where chum and chinook salmon spawned (Gharrett et al. 1987). Central Alaska, including much of the Yukon River system, was not covered with ice during the last major glaciation (Guthrie 1966). In this scenario, Alaskan and Pacific Northwest populations may have been separated for the latter part of the Pleistocene, as well as the Quaternary, with several mtDNA lineages surviving in each area.

In summary, intraspecific mtDNA sequence divergences were low in chum and chinook salmon among the study locations. Different haplotypes occurred in chinook salmon in the Yukon/Kenai rivers and the Pacific Northwest. The number of chum salmon haplotypes was higher in the Yukon River than in the Pacific Northwest. The most common chum haplotype was shared between the Yukon River and the Pacific Northwest.

Our PCR procedure permits efficient assessment of mtDNA haplotype frequencies. It allows detection of variation across different parts of the mtDNA molecule and does not require the time and effort of methods involving purification of mtDNA, Southern blotting, and the use of radioactive isotopes. With the development of nuclear DNA primers, the procedure could be expanded to include the nuclear genome. The patterns of variation that we observed suggest that analysis of mtDNA with this procedure could support stock-separation efforts.

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Appendices

APPENDIX 1. Restriction enzymes used in the analysis of the PCR products (ND-1, ND-3/4, ND-5/6, D-loop) of salmon mtDNA. The enzymes that detected intraspecific variation are identified in Appendix 2.

Chinook D-loop	Chum ND-3/4	Chum/chinook ND-1	Chum/chinook ND-5/6
Apo I	*Acc* I	*Acc* I	*Acc* I
Ase I	*Ase* I	*Ase* I	*Alu* I
*Bsa*J I	*Ava* II	*Ava* II	*Ase* I
Bsl I	*Bbs* I	*Bbs* I	*Bsa*J I
*Bsp*1286 I	*Bgl* I	*Bgl* I	*Bsp*1286 I
*Bst*N I	*Bsa*J I	*Bsa*J I	*Bst*N I
*Bst*U I	*Bsm*A I	*Bsm*A I	*Bst*U I
Dde I	*Bsp*1286 I	*Bsp*1286 I	*Dde* I
Dpn II	*Bsr* I	*Bsr* I	*Dpn* II
Dra I	*Bst*N I	*Bst*N I	*Dra* I
Hae III	*Bst*U I	*Bst*U I	*Hae* III
Hha I	*Dde* I	*Dde* I	*Hha* I
Hinc II	*Dpn* II	*Dpn* II	*Hinc* II
Hinf I	*Dra* I	*Dra* I	*Hinf* I
Hph I	*Hae* III	*Hae* III	*Hph* I
Mse I	*Hha* I	*Hha* I	*Msp* I[a]
Msp I	*Hinc* II	*Hinc* II	*Nci* I
Nci I	Hind III	*Hind* III	*Rsa* I
Rsa I	*Hinf* I	*Hinf* I	*Sau*96 I
Ssp I	*Hph* I	*Hph* I	*Taq* I
Taq I	*Msp* I	*Msp* I	
	Nci I	*Nci* I	
	Rsa I	*Rsa* I	
	*Sau*96 I	*Taq* I	
	Taq I	*Xba* I	
	Xba I		

[a]This was used only with chinook salmon.

APPENDIX 2. Salmon mtDNA restriction fragment sizes and patterns for enzymes that detected intraspecific variation. For example, there were three fragment patterns (A, B, C) from digestion of chum salmon ND-5/6 with *Ase* I. Pattern A had fragments 1427 and 1081 base pairs long, pattern B had fragments 1427, 818, and 440 base pairs long, and patern C had fragments 871, 818, 634, and 440 base pairs long. The occurrence of fragment patterns in composite haplotypes is described in Tables 3 and 4.

Chum salmon						Chinook salmon					
mtDNA segment	Restriction enzyme	Fragment size	Pattern			mtDNA segment	Restriction enzyme	Fragment size	Pattern		
ND-5/6	*Alu* I	1256	A	—	—	ND-5/6	*Bst*U I	1712	—	C	—
		769	A	C	—			1169	A	—	—
		552	A	—	—			731	A	C	—
		515	—	C	—			474	A	—	—
	Ase I	1427	A	B	—						
		1081	A	—	—	ND-1	*Ase* I	2072	—	C	—
		871	—	—	C			1072	A	—	—
		818	—	B	C			818	A	—	—
		634	—	—	C		*Hae* III	631	A	C	—
		440	—	B	C			450	—	C	—
	Dpn II	1432	B	C	—			411	A	C	—
		540	B	C	—			329	A	—	—
		457	—	C	—			295	A	C	—
	Rsa I	1833	—	C	—		*Rsa* I	664	A	C	—
		1540	B	—	—			522	—	C	—
		413	B	C	—			353	A	—	—
								346	A	C	—
	*Sau*96 I	652	B[a]	C	D[a]			310	A	C	—
		516	B	C[a]	D			300	A	—	—
		489	—	—	D						
		440	B	C	—		*Ava* II	867	A	—	—
								566	—	C	—
	BsaJ I	955	B	—	—			448	A	C	—
		867	B	C	—			386	—	C	—
		722	—	C	—			367	A	C	—
		469	—	C	—			308	A	C	—
		436	B	C	—			291	A	C	—
	Taq I	762	B	C	—						
		716	B	C	—	D-loop	*BsaJ* I	538	A	—	D
		535	B	C	—			479	—	C	—
		493	B	C	—			439	A	C	—
		444	—	C	—			388	A	C	D
							*Bst*N I	547	A	C	—
ND-3/4	*Rsa* I	540	B	C	—			465	A	—	—
		486	B	C	—			445	A	C	—
		467	—	C	—			350	—	C	—
		405	B	C	—			325	—	C	—
	*Bst*U I	1404	B	C	—			308	A	C	—
		624	—	C	—						
		514	B	C	—						
		371	B	C	—						

[a]There are two fragments of equal size in these patterns.

Shape of Zooplankton and Retention in Filter-Feeding: A Quantitative Comparison between Industrial Sieves and the Branchial Sieves of Common Bream (*Abramis brama*) and White Bream (*Blicca bjoerkna*)

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Industrial sieves retained all cycloid copepods with a width larger than their mesh size, but *Daphnia*, with a width up to 1.4 times the mesh size, still passed through them. *Daphnia* have a lower depth/width ratio than copepods (0.599 and 0.882, respectively). Therefore, *Daphnia* could pass through the square meshes diagonally. In filter-feeding experiments with common bream (*Abramis brama*), the smallest retained copepods correspondingly were about 35% less wide than the smallest retained *Daphnia*. White bream (*Blicca bjoerkna*) did not retain copepods smaller than *Daphnia*. In the reducible-channel model of filter-feeding, particles are retained in the channels between the medial gill rakers. The mesh size can be reduced by lowering the lateral rakers into these channels. We calculated that zooplankton depth is the critical size parameter in reduced channels and zooplankton width in unreduced channels. We found that white bream was feeding with unreduced channels and common bream with reduced channels. The depth/width ratio (35% lower in *Daphnia* than in copepods) therefore explains the difference in retention of copepods and *Daphnia* by common bream whereas no such difference was expected for white bream. The shape of zooplankton thus affects the trophic segregation and the exploitation of food resources by fish.

Les tamis industriels retiennent tous les copépods cycloïdes dont la largeur est supérieure à la taille des mailles, mais des *Daphnia* dont la largeur pouvait atteindre 1,4 fois la taille des mailles pouvaient les traverser. Les *Daphnia* ont un rapport épaisseur-largeur inférieur à celui des copépodes (valeurs respectives de 0,599 et 0,882) et peuvent donc passer dans la diagonale des mailles carrées. Au cours d'essais portant sur l'alimentation par filtrage des brèmes communes (*Abramis brama*), les plus petits copépodes retenus étaient de 35 % environ moins larges que les plus petites *Daphnia*. Les brèmes blanches (*Blicca bjoerkna*) ne retenaient pas de copépodes plus petits que des *Daphnia*. Dans le modèle à canal réductible des filtreurs, les particules sont retenues dans les canaux entre les peignes branchiaux médians. La taille des mailles peut être réduite en abaissant les peignes branchiaux latéraux dans les canaux. Nous avons calculé que, dans les canaux réduits, le paramètre de taille critique est l'épaisseur des organismes zooplanctoniques tandis que dans les canaux non réduits, ce paramètre est la largeur des organismes. Nous avons aussi trouvé que les brèmes communes se nourrissent en réduisant ces canaux tandis que les brèmes blanches ne le font pas. Le rapport épaisseur-largeur (35 % inférieur chez les *Daphnia* par rapport au copépodes) explique donc l'écart entre la rétention des copépodes et des *Daphnia* chez la brème commune, tandis qu'aucun écart de ce genre ne devrait exister chez la brème blanche. La forme des organismes zooplanctoniques influe donc sur la ségrégation trophique et l'exploitation des ressources alimentaires par les poissons.

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The effective mesh size or retention ability (ability to retain small particles) of the branchial sieve is an important parameter for fish that forage on small particles. This parameter can be determined from the size selectivity of filter-feeding fish in laboratory experiments. One problem in generalizing the results of such experiments, however, is the influence of the shape of zooplankton on retention by the branchial sieve. In previous studies of filter-feeding by coregonids and clupeids, the average of length, width, and depth of the zooplankton was used as a critical zooplankton size parameter (Wright et al. 1983; Drenner et al. 1984; Mummert and Drenner 1986; Gibson 1988; MacNeill and Brandt 1990), assuming a random orientation of the zooplankton when they meet the comblike branchial sieve in these fishes. Wright and O'Brien (1984) assumed that the average of width and depth is critical. Wright et al. (1983) suggested that the appendages of the zooplankton may play a role in retention. Mummert and Drenner (1986) found that *Pediastrum* (a circular, flat colony of green algae) was removed by *Dorosoma cepedianum* at the same rate as microspheres with a diameter four times smaller. This implies

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that the depth, not the width, of *Pediastrum* is the important factor for retention by *D. cepedianum*.

To better understand the retention ability of the branchial sieve, the shape of zooplankton clearly deserves more attention. We designed a separation system with industrial sieves (Fig. 1) in order to sieve live zooplankton and to study the relationship between zooplankton shape and mesh size. The results of these sieving experiments were then compared with the results of laboratory experiments with filter-feeding common bream (*Abramis brama*) and white bream (*Blicca bjoerkna*).

Materials and Methods

Zooplankton were collected from April to July 1990 in the Dutch Lake Tjeukemeer using hoop nets (mesh size 250 μm) with a collecting bottle at the end. The velocity of the boat was approximately 1 $m \cdot s^{-1}$ and each haul lasted approximately half a minute. The zooplankton community is composed of cycloid copepods and cladocerans. Dominant cladocerans were *Daphnia hyalina, Daphnia cucullata, Bosmina coregoni, Bosmina longirostris, Chydorus sphaericus*, and *Leptodora kindtii* (cf. de Nie et al. 1980). In our experiments, we distinguished two groups of zooplankton: the cycloid copepods and the *Daphnia* species. The other species were not considered.

Separation into Size Classes and Measurement of Zooplankton

The zooplankton were poured into buckets and the debris (algae, sand, and dead zooplankton) was allowed to settle. Floating zooplankters, with an air bubble under their carapace, were removed by skimming them off the surface. The remaining suspension was poured gently into a funnel-shaped storage tank (Fig. 1A) and allowed to settle again for about 30 min. The zooplankton suspension then flowed from the tank to the uppermost of a stack of sieves through a tube with a tap. The inlet of the tube was above the floor of the storage tank to avoid sucking in debris. To avoid air contact, the stack of sieves was permanently immersed in a large tank of water (Fig. 1A). We used seven stainless steel laboratory test sieves (Endecotts Ltd., London, diameter 200 mm, height 50 mm) arranged with diminishing mesh size (850, 600, 425, 355, 300, 250, and 150 μm, respectively) from top to bottom. The sieves are made of interwoven wires in a square pattern (Fig. 1B). Each sieve was provided with an outlet tap. The stack was topped with a 15-cm-high rim to prevent overflowing due to the pressure difference over the sieves. Water and nonretained particles flowed from the bottom of the stack into the large tank. During the filtration trials, all the outlet taps of the sieves were closed. Once the zooplankton storage tank was empty, the flow consisted of tap water. The downward flow velocity in the stack averaged approximately 0.5 $mm \cdot s^{-1}$ ($\approx$15 $mL \cdot s^{-1}$). A higher flow velocity would have resulted in overflowing because of the resistance offered by the sieves, especially when they became clogged. Live zooplankton can probably swim against such a flow; therefore, we tried to attract the zooplankton toward the bottom of the stack with a lamp under the large tank (Fig. 1A). The top of the stack was darkened with black plastic. Each filtration trial continued for at least 12 h, usually at night. The continuous flow of water guaranteed fresh, oxygenated water for the very dense zooplankton population (1×10^4 to $2 \times 10^4 \cdot L^{-1}$) between the sieves; the volume of water in the stack of sieves was replaced every 10 min. Following filtration,

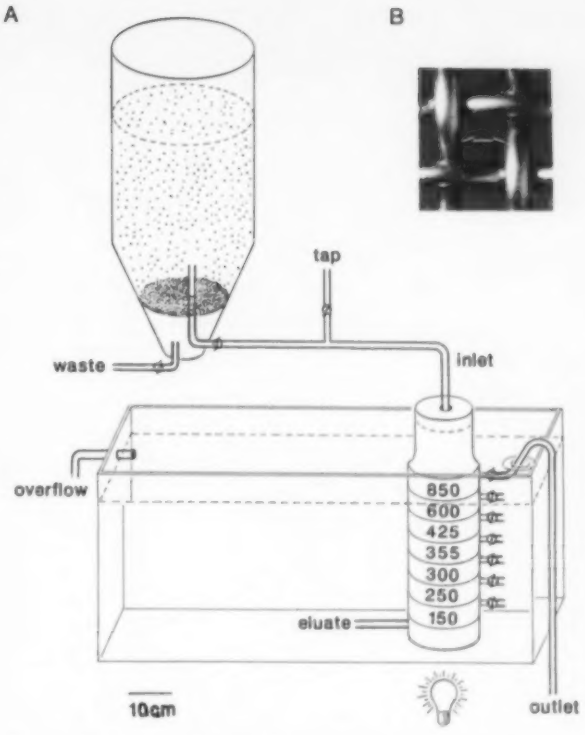

FIG. 1. (A) Experimental setup for filtering live zooplankton through industrial sieves. The suspension of zooplankton flows from a storage tank through a stack of industrial sieves with diminishing mesh size (μm). (B) Detail of an industrial sieve. Mesh size is measured as indicated.

zooplankton from a particular sieve were collected by opening the outlet tap and siphoning the water with live zooplankton into a bucket while increasing the flow velocity of the tap water in order to prevent reverse flow in the stack of sieves.

Zooplankton samples were preserved in 4% formalin. The samples were analyzed by counting the zooplankton per species and measuring individual lengths and widths to the nearest 0.03 mm using an ocular micrometer. Measurements are illustrated in Fig. 2B. If samples contained more than a few hundred specimens, a subsample was taken using a whirling apparatus (Kott 1953). Zooplankton lengths were measured following Vijverberg and Richter (1982a, 1982b), Wright et al. (1983), and Wright and O'Brien (1984). For copepods, the length of the cephalothorax was measured. For *Daphnia*, the tail and helmet, when present, were subtracted from the length. On a microscope slide, zooplankton always lie on their flattest side. In this orientation, zooplankton width equals the minimal distance between two parallel tangents on either side of the body (Fig. 2B). The depth/width (d/w) ratio was measured with the same zooplankton, floating in a small tray with formol, using an ocular micrometer.

Because of the sequential arrangement of the sieves, the size–frequency distribution of zooplankton passing through a particular sieve is dependent on the sieves above it (with larger mesh size). These sieves set an upper boundary for the size range of zooplankton in each sieve sample. In this setup, the upper boundary is therefore very useful for interpretation of the

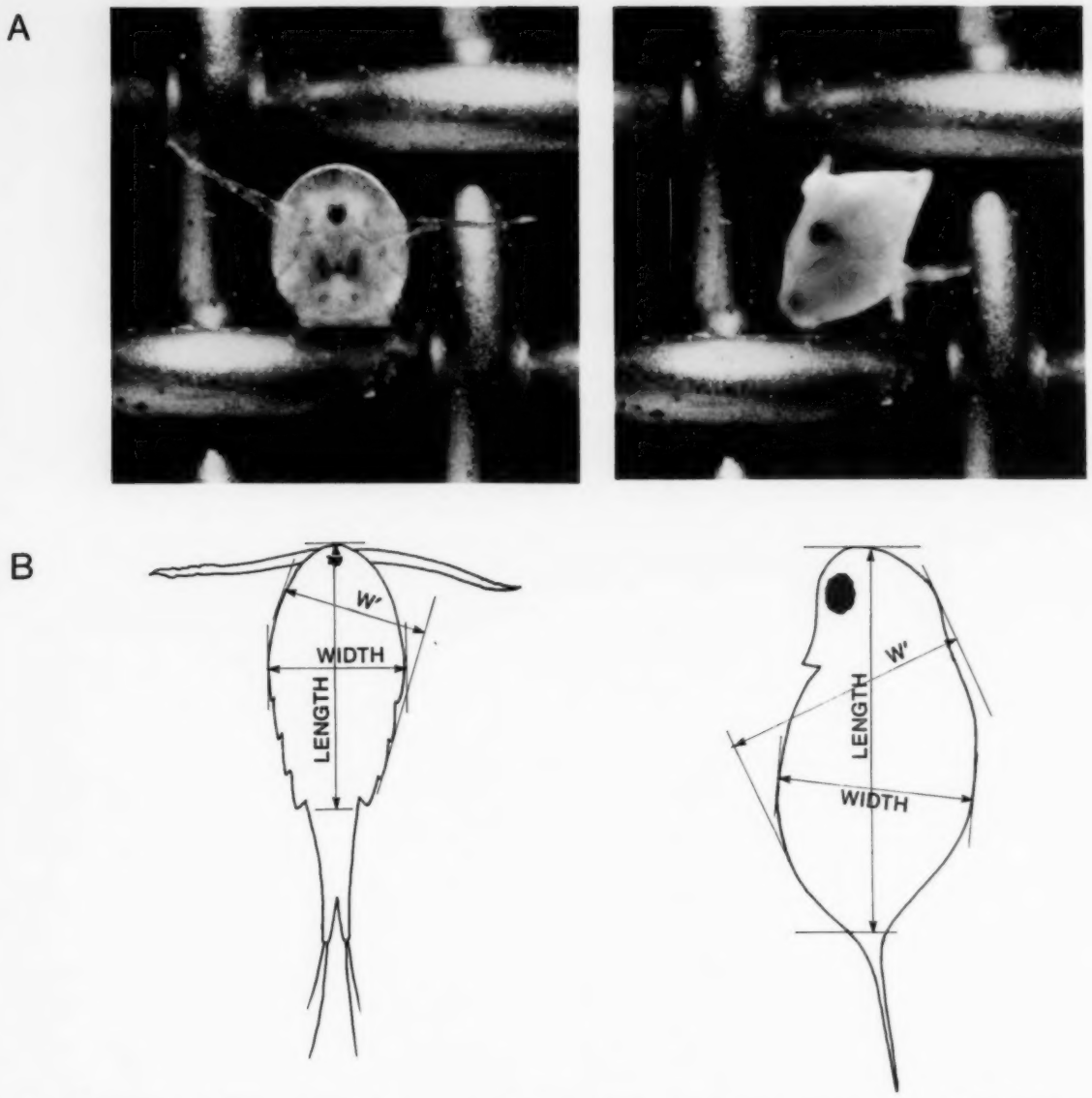

FIG. 2. (A) Photographs of an industrial sieve with a copepod and a *Daphnia*. Live zooplankton were embedded in 2% ultra low gelling agarose, which permitted easy handling and a view of all sides of the plankton. This head-on view shows that the depth of *Daphnia* equals about 60% of its width (the *d*/*w* ratio is approximately 0.6). The copepod is almost circular in this view (the *d*/*w* ratio is approximately 0.9). Because of this difference in *d*/*w* ratio, *Daphnia* can pass square meshes diagonally whereas copepods cannot. (B) Measurement sites of width and length of a copepod and a *Daphnia*. Two sets of parallel tangents on either side of the body of the zooplankton are shown. W' indicates a wrong way to measure width. The set with the shortest distance between the tangents is defined as the plankton width. By definition, width corresponds to the smallest mesh size that the zooplankton can pass through. Note that in *Daphnia*, width is usually not perpendicular to length.

results but the lower boundary is not. The lower boundaries in the sieve samples are influenced by clogging, back-swimming of the zooplankton, and possibly by zooplankton orientation. These effects cannot be distinguished. Therefore, the lower boundaries are not very useful for interpretation of the results.

Filter-feeding Experiments

Common bream (standard length (SL) 17.6 cm) and white bream (SL 17.2 cm) were each kept in a tank with a known volume of water (V). A suitable mixture of zooplankton size classes from the industrial sieve system was added to the water, which marked the start of the experiment (t = 0 h). At eight locations in each tank, plankton samples of about 0.15 L each were taken using a sampling tube (Hoogenboezem et al. 1993). The eight samples were lumped and preserved in 4% formalin. Sample volume was measured to the nearest 5 mL. The fish were then allowed to feed for 6 h. When feeding on zooplankton, common bream and white bream can use either particulate intake or gulping. Because the experiments were conducted in

TABLE 1. Length, width, and depth measurements (mean ± SD) of copepods and *Daphnia* in samples from six industrial sieves. The average of width divided by length is used in the filter-feeding experiments to calculate width from length. The depth/width ratio was measured separately, but using the same plankton. Note the slightly allometric growth of the length/width ratio in *Daphnia*. n = number of measured zooplankton.

Sieve mesh size (μm)	Copepods			*Daphnia*		
	Length (mm)	Width (mm)	n	Length (mm)	Width (mm)	n
600	0.832 ± 0.147	0.414 ± 0.087	26	1.285 ± 0.146	0.646 ± 0.119	102
425	0.834 ± 0.148	0.413 ± 0.079	63	1.200 ± 0.111	0.598 ± 0.087	101
355	0.627 ± 0.112	0.302 ± 0.046	99	0.977 ± 0.177	0.420 ± 0.082	49
300	0.597 ± 0.056	0.281 ± 0.044	100	0.752 ± 0.134	0.326 ± 0.062	27
250	0.501 ± 0.061	0.238 ± 0.031	102	0.646 ± 0.105	0.279 ± 0.052	53
150	0.360 ± 0.045	0.184 ± 0.027	38	0.593 ± 0.045	0.268 ± 0.028	113
Average width/length	0.487 ± 0.0030		428	0.468 ± 0.0032		445
Average depth/width	0.882 ± 0.072		20	0.599 ± 0.066		20

the dark the fish were forced to gulp; prey were not selected visually. At the end of the experiment (t = 6 h), another eight samples were taken. The zooplankters were measured and grouped in length classes of 0.06 mm. Their width was calculated from their length using the empirical relationship shown in Table 1. Zooplankton size classes with less than five specimens at t = 0 h were not included in the analysis.

Zooplankton Retention by the Branchial Sieve

Calculation of Zooplankton Retention from Feeding Experiments

The difference between the samples at t = 0 h and at t = 6 h in density of a size class of zooplankton represents the fraction of zooplankton from that size class that has been eaten. The experiments were carried out in darkness in order to avoid visual selection and particulate intake of prey and hence to induce continuous, random gulping by the fish. The plankton distribution was kept random with an air stone and by movements of the fish. Under these conditions the plankton density will decrease exponentially. Whether this exponential decrease in density is slow or rapid for a certain size class of zooplankton depends on the filtering rate of the fish (litres per hour) and on the retention percentage for the particular size class of zooplankton. This rate of decrease in density of a particular size class is called the clearance rate (after Smith 1989). The exponential decrease in density of a particular size class is (cf. Drenner et al. 1984; Hoogenboezem et al. 1993)

$$(1)\quad D_t = D_0 e^{-(\mathrm{CR}\cdot t)/V}$$

where D_t = density of a particular zooplankton size class at time t, D_0 = initial density of the same zooplankton size class (t = 0), CR = clearance rate (litres per hour), t = duration of the experiment (hours), and V = tank volume (litres). If retention is 100%, the clearance rate equals the filtering rate of the fish. The filtering rate is therefore estimated in each experiment as the maximal clearance rate that is found. The retention percentage (R) of each size class of zooplankton equals the clearance rate divided by the filtering rate (FR) (cf. Smith 1989):

$$(2)\quad R = \mathrm{CR} / \mathrm{FR}$$

and the combination of equations 1 and 2 yields

$$(3)\quad \frac{\ln (D_0/D_t)\cdot V}{\mathrm{FR}\cdot t}.$$

When D_t is 0, this formula yields $R = \infty$. In these cases, we assumed that R = 100%.

Theoretical Retention of Zooplankton

We assumed that during successive gulps, the water flow would reorient the zooplankton until its length axis lay parallel to the channels. We used the reducible-channel model (Hoogenboezem et al. 1991) to obtain theoretical retention values (Fig. 3A). In this model the channels between the medial raker cushions are the site of prey retention. The channel width equals the mesh size. The roof of the channels is formed by the palatal organ. The channels are mostly circular in cross section (Fig. 3B); therefore the diagonal of the channel and its width are equal. This means that zooplankton width is the critical size parameter.

Channel widths (CW) were obtained using the allometric equations CW = −0.104 + 0.00462·SL for common bream and CW = 0.038 + 0.00330·SL for white bream (van den Berg et al. 1992) where SL = standard length (millimetres). We found that CW is 0.71 mm for the common bream and 0.61 mm for the white bream. CW was measured only in the middle of the second gill arch. At this position, CW is maximal. In fact, there is a range of channel widths (between 60 and 100%) throughout the branchial sieve (van den Berg et al. 1992). We assumed that the retention curve is sigmoid running from 0% retention at 0.6·CW to 100% retention at CW. This is the theoretical retention curve for unreduced, circular channels.

In the reducible-channel model, the mesh size of the branchial sieve can be reduced by lowering the lateral rakers into the medial channels (Hoogenboezem et al. 1991), turning them into reduced channels (Fig. 3A and 3B). In reduced channels the retention depends on the d/w ratio and on the ratio of the lateral raker radius and the channel radius (Fig. 3C). With a geometric analysis of the reduced channels (see Appendix), we predict that zooplankton depth is the critical size parameter when the d/w ratio is above 0.58 ($1/\sqrt{3}$). The d/w ratio of both copepods and *Daphnia* is above this value (Table 1); therefore, zooplankton depth is the critical size parameter for reduced channels. We estimated that the diameter of the lateral raker tip is 20% of the medial channel diameter. The resulting formula for the mesh size of the reduced channels is

$$(4)\quad \frac{0.40\cdot \mathrm{CW}}{d/w}.$$

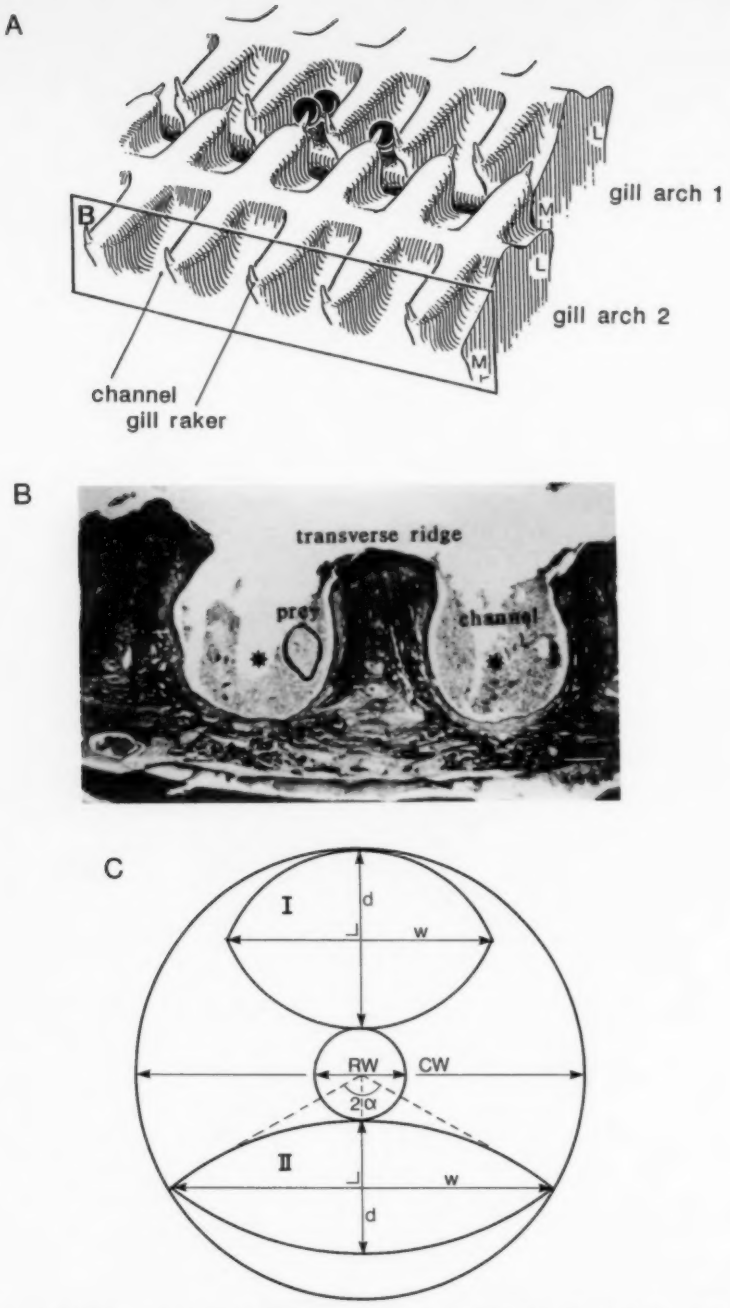

FIG. 3. (A) Reducible-channel model for filter-feeding. The mesh size of the branchial sieve is reduced by depressing the lateral rakers into the medial channels. (Adapted from Hoogenboezem et al. 1993) (B) Longitudinal section (Crossmon staining) of a branchial arch of a common bream. The medial channels appear in cross section and are roughly circular. When the channels are reduced the tips of the lateral rakers of the adjacent arch are positioned as indicated by the stars. A raker cushion, or channel wall, is indicated as a transverse ridge. (Adapted from Hoogenboezem et al. 1991) (C) Mathematical model of a reduced channel. A *Daphnia*-shaped particle can be retained in two ways, according to depth, d (method I), or according to width, w (method II). The mode of retention depends on the ratio of lateral raker tip width (RW) and channel width (CW) and on the d/w ratio. The value of RW/CW is estimated to be 0.20. In this case, particles with $d/w > 0.50$ will be retained by method I and particles with $d/w < 0.50$ by method II (see Appendix). Angle α is used in the calculations in the Appendix.

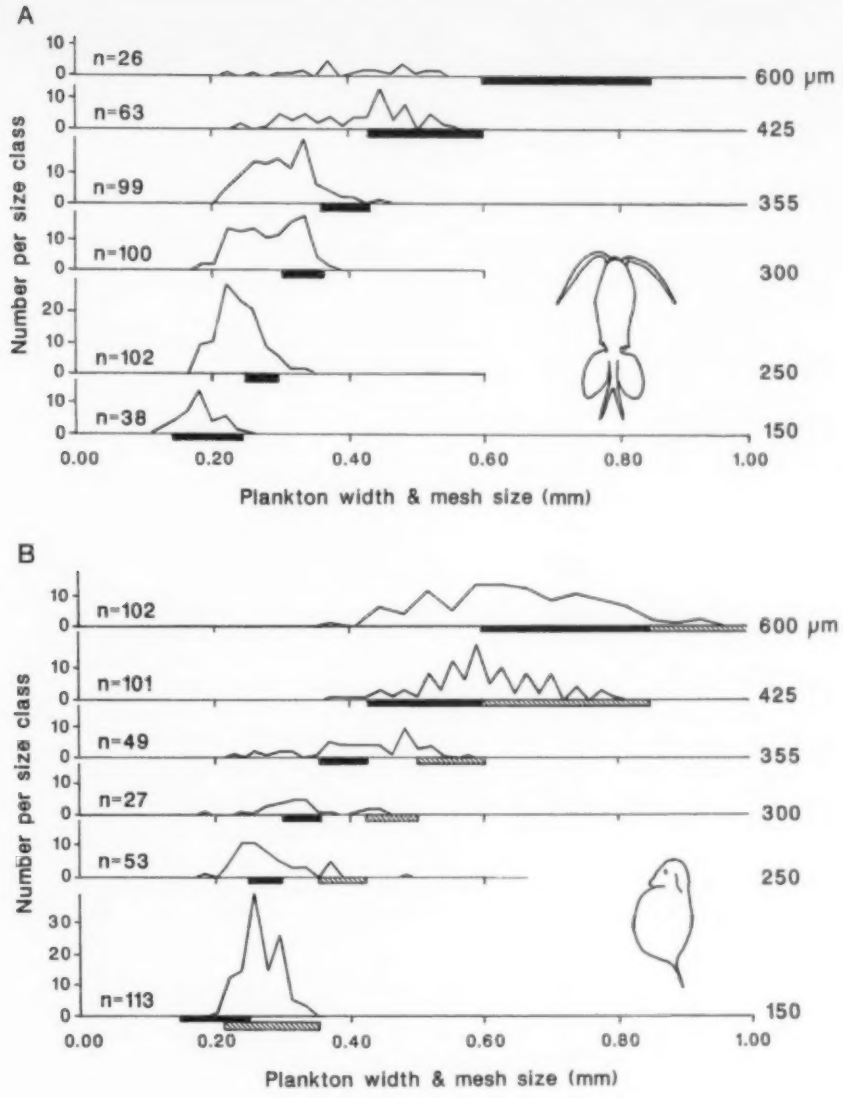

FIG. 4. Width–frequency distributions of zooplankton in samples from six subsequent industrial sieves (600, 425, 355, 300, 250, and 150 µm). The black bars indicate the expected zooplankton size range. The upper boundary is the mesh size of the sieve above the sample sieve; the plankton did pass through this sieve. The lower boundary is the mesh size of the sample sieve itself; the plankton did not pass through this sieve. (A) Copepods. The maximum widths correspond with the upper boundaries of the expected size ranges, indicating that width is the critical size parameter for copepods. (B) *Daphnia*. The hatched bars indicate the expected zooplankton size range when the diagonal of the square meshes is used as mesh size (see Discussion). The maximum widths of *Daphnia* correspond to the upper boundaries of these "diagonal" expected size ranges. Width is the critical size parameter for *Daphnia*, but, unlike copepods, *Daphnia* can pass the sieve diagonally, due to their three-dimensional shape.

For copepods and *Daphnia*, a sigmoid curve was drawn between 60 and 100% of this value. These are the theoretical retention curves for reduced, circular channels. Note that in unreduced channels, zooplankton width is the critical size parameter whereas in reduced channels, zooplankton depth is the critical size parameter.

Results

The width to length ratio is 0.487 for copepods and 0.468 for *Daphnia* (Table 1). The *d*/*w* ratio is 0.882 for copepods and 0.599 for *Daphnia* (Table 1). Because these values of the *d*/*w* ratio are larger than 0.58, zooplankton depth is the critical zoo-

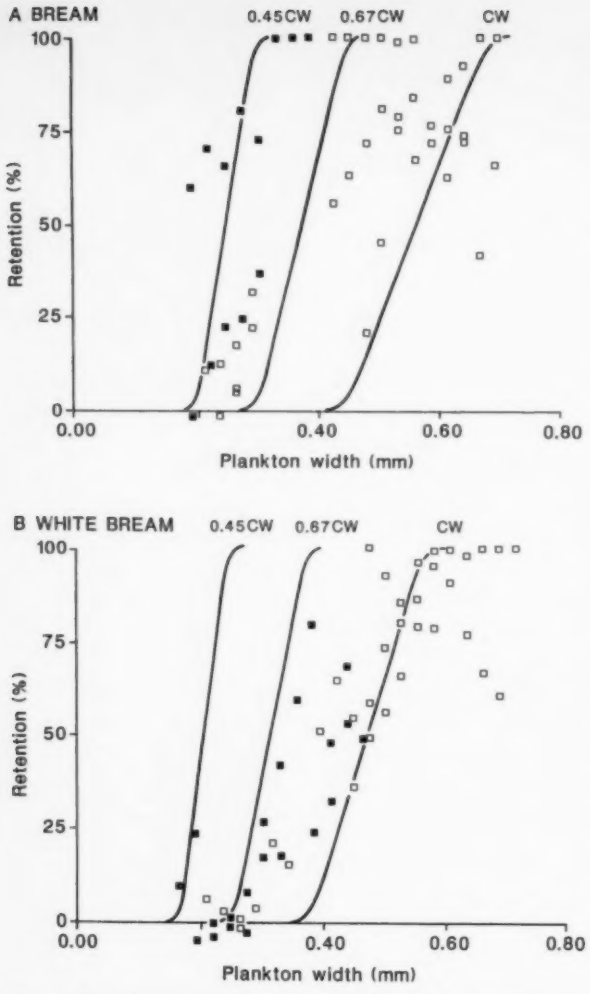

FIG. 5. Retention percentage versus plankton width in (A) common bream (SL = 17.6 cm) and (B) white bream (SL = 17.2 cm). Data are compiled from three filter-feeding experiments. Note that the maximum width of the copepods (solid squares) is 0.4 mm and of *Daphnia* (open squares) is 0.7 mm. The theoretical retention curve for unreduced channels is indicated (CW, channel width). The theoretical retention curves for reduced channels are indicated with 0.45 CW for copepods and 0.67 CW for *Daphnia*. The multiplication factors of CW are calculated as 0.40 divided by the *d*/*w* ratio (0.882 for copepods and 0.599 for *Daphnia*) because depth is the critical size parameter.

plankton size parameter in reduced channels for both copepods and *Daphnia*. Using equation 4, we find that the theoretical mesh size of reduced channels is 0.45·CW for copepods and 0.67·CW for *Daphnia*.

It is expected that the size range of zooplankton within a sieve sample is determined by the mesh size of the sample sieve itself and of the sieve above it (with larger mesh size). The mesh size of the sample sieve is the lower boundary of the expected zooplankton size range; the mesh size of the upper sieve is the upper boundary. The upper boundary is absolute (i.e., zooplankton did pass through the upper sieve). The lower boundary is not absolute (i.e., zooplankton that did not pass through the sample sieve may still be able to do so).

The average copepod length in each sieve sample (which, in our standard ecological measurements, excludes the tail) generally equals about 1.5 times the upper boundary of the expected size range (Table 1; e.g., average length 360 μm in the 150-μm sample, with an upper boundary of 250 μm). *Daphnia* length averages almost 2.5 times the upper boundary. Therefore, length clearly is not the critical parameter.

The measured zooplankton width–frequency distribution and the corresponding expected size range of each sieve sample are indicated in Fig. 4. There is a good fit between the maximal widths of copepods encountered in the sieve samples and the expected upper boundaries (Fig. 4A). The maximal widths of *Daphnia*, however, exceed the upper boundaries by up to 40% (Fig. 4B). Apparently, width is the critical size parameter for copepods. As expected, the lower boundaries are not distinct, for either of the zooplankton. This could be caused both by clogging of the sieves and by the possibility that small zooplankters remain in or swim back to larger sieves. *Daphnia* smaller than 0.18 mm in width (first instar) do not occur, nor do copepods larger than 0.56 mm in width, which probably explains the deviation in size–frequency distribution in the smallest and largest sieves.

Zooplankton retention by common bream and white bream is plotted versus plankton width in Fig. 5. The results of three experiments are combined in each graph. The initial plankton density was between 100 and 500·L^{-1} in each experiment. Note that the maximum width of the copepods is 0.4 mm and of *Daphnia* is 0.7 mm. The copepod retention data for common bream (Fig. 5A) correspond well with the theoretical curve for reduced channels. The data for *Daphnia* are more scattered, but the highest retention values from each size class correspond closely to the theoretical curve for reduced channels. The wide spectrum of retention percentages of *Daphnia* during the 6-h experiments suggests that at the initial high plankton density the common bream was feeding with unreduced channels and later switched to reduced channels. This switch was previously observed in common bream by Hoogenboezem et al. (1991). In white bream (Fig. 5B) in the size range where copepods occurred, we found no clear difference in retention of copepods and *Daphnia*. Data for white bream correspond most closely to the theoretical curve for unreduced channels, although retention is slightly better than expected. The increase in retention from 0 to 100% is more gradual than predicted.

The average filtering rates of common bream and white bream were 27.5 and 23 L·h^{-1}, respectively. The maximal clearance rate for copepods was about 80% of these filtering rates for both common bream and white bream.

Discussion

In the industrial sieve experiments the maximal copepod width was equal to the mesh size of the sieve through which it passed, but the maximal *Daphnia* width exceeded the mesh size of the sieve through which it passed by about 40% (Fig. 4). This difference can be explained if one considers the three-dimensional shape of these organisms. The *d*/*w* ratio is about 0.9 in copepods and 0.6 in *Daphnia* (Table 1; Fig. 2a). Similarly, Wright and O'Brien (1984) found a *d*/*w* ratio of about 0.80 in *Mesocyclops edax* and 0.38 in *Daphnia parvula*. Because of its low *d*/*w* ratio, *Daphnia* can pass through square meshes diagonally (Fig. 2A). We calculated the expected plankton size ranges using the diagonal mesh size (hatched bars in Fig. 4B), which equals $\sqrt{2}$ times the original mesh size. As

expected, the maximum widths of *Daphnia* correspond to the upper boundaries of these "diagonal" expected size ranges.

The relationship between plankton width and the mesh size that it can pass through is very close. Apparently the antennae of copepods and *Daphnia* do not prevent the plankton from passing through the sieves, even at the very low experimental flow velocities. Kerfoot (1978) described the "dead-man response" in *B. longirostris*. When it perceives a predator, it folds its antennae into its carapace and passively sinks away from the predator, thus avoiding mechanoreceptive perception of its exact location. This phenomenon may also explain why the antennae do not influence retention.

Both the copepod and *Daphnia* retention data for common bream correspond closely to the reduced channel (RC) curves. Therefore, the reducible-channel model appears to be valid for common bream (cf. Hoogenboezem et al. 1991). The common bream retained copepods of about 65% of the width of the smallest retained *Daphnia*. There is a similarity with the industrial sieves; *Daphnia* are more difficult to retain than copepods of the same width because of their low *d*/*w* ratio. The maximal clearance rate for copepods was about 80% of the filtering rate. This could indicate that the high escape velocity of copepods (Kerfoot 1978: 35 $cm \cdot s^{-1}$) has some influence on retention by the fishes. Janssen (1976) showed that *Daphnia* do not escape at all whereas copepods are negatively rheotactic. The different retention of copepods and *Daphnia* by common bream therefore cannot be caused by this difference in escape behaviour. If it were, we would expect copepods to be retained less well than *Daphnia* whereas we found the opposite. Furthermore, we would expect the larger, quicker copepods to be retained less well than the small ones. Again, we found the opposite (Fig. 5).

The retention data for white bream correspond most closely to the unreduced channel (URC) curve. In white bream, we found only a minor difference in the retention of copepods and *Daphnia*, as was expected theoretically for unreduced channels. The white bream probably did retain zooplankton in its channels but did not reduce its channels. Hoogenboezem et al. (1991) showed that the lateral gill rakers of common bream have a musculus abductor branchiospinalis capable of abducting (lowering) the lateral rakers into the medial channels. This tiny muscle is not present in white bream (unpubl. data), indicating that white bream are unable to reduce their channels. This clearly corroborates the present results.

An important conclusion is that zooplankton depth is the critical size parameter only when the channels are reduced (see Appendix). Therefore, both the shape of the biological sieve and the shape of zooplankton should be examined in order to predict retention accurately.

We have predicted that in unreduced channels, zooplankton width is the critical size parameter because the channels are mostly circular in cross section (Fig. 3B). The roof of the channels, however, is formed by the muscular palatal organ (Sibbing et al. 1986; Sibbing 1991). Possibly, the roof is not circular. This means that zooplankton depth could play a role in unreduced channels as well, which might explain the slightly better retention of copepods by white bream (Fig. 5B).

In previous experiments (Hoogenboezem et al. 1993), formalin-preserved zooplankton were sieved by pouring the zooplankton over an industrial sieve and rinsing the sieve with water. In this way, a wider size range of zooplankton was retained than by using the present method and the relationship between mesh size and particle size was less clear. The orientation of the dead plankton is probably more random than that of live ones. In our opinion, the present underwater method with live zooplankton is more similar to the actual events that occur in the branchial sieve. Although the orientation of the zooplankters entering a channel is unknown, plankton width and depth are more important than length. The forces exerted by the water during gulping will reorient a prey until its length axis is parallel to the direction of flow and to the channel walls. Drost et al. (1988) found that carp larvae always take in elongated prey items with their length parallel to the flow because of the steep velocity gradients within the flow. They concluded that prey width is critical. The lack of knowledge of flow pattern and prey orientation in the branchial sieve stresses the need for measuring water flow profiles in live fish. Unfortunately, this requires highly complicated experiments (e.g., Sanderson 1991). It is likely that prey orientation is more random when the branchial sieve is comb-shaped, as in many clupeids and coregonids. In that case, a combination of zooplankton size parameters is probably more meaningful than just zooplankton width or depth (Wright et al. 1983; Drenner et al. 1984; Mummert and Drenner 1986; Gibson 1988; MacNeill and Brandt 1990).

The complex and variable shape of zooplankton makes it difficult to predict retention accurately. Experiments have been performed with mixtures of zooplankton and microspheres of different sizes (Drenner et al. 1984; Mummert and Drenner 1986). The well-defined diameter of microspheres makes the assessment of the mesh size of the branchial sieve comparatively easy. During natural functioning, however, the branchial sieve is not retaining spheres but zooplankton. The relationship between the shape of zooplankton and of the branchial sieve is crucial to understand filter-feeding. We found that different zooplankton size parameters may be critical depending on the species of fish and zooplankton and on the filtering mode. In unreduced channels, zooplankton width is critical. In reduced channels, zoooplankton depth is critical, provided that the *d*/*w* ratio is higher than 0.58. The metrical relationships between length, width, and depth of the zooplankton are, therefore, essential to interpret filter-feeding experiments in fish. In fact, the differential retention of copepods and *Daphnia*, which only occurs in common bream, strongly supports the reducible-channel model (Hoogenboezem et al. 1991). Our results have implications for the zooplankton as well. Differently shaped species will have a different chance of predation depending on the shape of the sieve of the predator. The flat shape of *Pediastrum*, for example, might be an adaptation to prevent ingestion by *D. cepedianum* (Mummert and Drenner 1986). The present study of the interaction between live zooplankton and sieves show that the shapes of both are essential to test theoretical filter-feeding models in order to understand filter-feeding in fishes. Such detailed knowledge is fundamental to trophic segregation and the exploitation of food resources by fish.

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Appendix

Square Meshes of the Industrial Sieves

Particles should fulfil two conditions to pass a square mesh diagonally: (1) the d/w ratio should not exceed $1/\sqrt{2}(\approx 0.71)$ and (2) the profile edges in the width direction should be able to pass the rectangular corners of the mesh. The angle of the profile edges should therefore be less than 90°. *Daphnia* meet both of these demands; copepods meet neither of them (Fig. 2A).

Mathematical Model for Retention in a Reduced Channel (Fig. 3C)

The channel is a circle with diameter CW; the lateral raker tip is a smaller circle in the centre of the channel, with diameter RW. The particle has depth d and width w. The contours of the particle are formed by two identical circle segments. A particle can be critically retained in two ways: (I) according to d, then $d = 0.5\text{CW} - 0.5\text{RW}$, or (II) according to w, with a complex relationship to CW. The relationship for method II is obtained using angle 2α, which is the angle between the contact points of a critical particle with the channel wall and the centre of the channel. It can be shown that

$$\cos \alpha = \frac{d + \text{RW}}{\text{CW}}$$

and

$$w = \text{CW} \sin \alpha$$

rewritten as

$$d/w = \frac{\cos \alpha - \text{RW/CW}}{\sin \alpha}.$$

With given ratios d/w and RW/CW the value of α can be determined iteratively; with α the value of d and w can be determined. If the value of d obtained in this way exceeds $0.5\text{CW} - 0.5\text{RW}$, method II is invalid and method I should be used. At the transition of methods I and II, both formulas should apply. It follows that

$$\cos \alpha = 0.5\text{RW/CW} + 0.5$$

and

$$d/w = \frac{0.5}{\sin \alpha}(1 - \text{RW/CW}).$$

Some values of RW/CW and d/w at this transition point are given in the following table:

RW/CW	d/w
0	$1/\sqrt{3} \approx 0.58$
0.1	$\sqrt{(9/31)} \approx 0.54$
0.2	$1/2 = 0.50$
0.5	$1/\sqrt{7} \approx 0.38$

For example, when RW/CW equals 0.10 and $d/w > 0.54$, method I is appropriate (retention according to d), and when $d/w < 0.54$, method II is appropriate (retention according to w). When d/w exceeds $1/\sqrt{3}$, method I is always appropriate, independent of the value of RW/CW.

Density-Dependent Geographic Distribution of Atlantic Cod (*Gadus morhua*) in the Southern Gulf of St. Lawrence

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Swain, D.P., and E.J. Wade. 1993. Density-dependent geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 50: 725–733.

We tested effects of population size on the geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence for age groups 3–8+ over the period 1971–90. Distribution was density dependent for all six age groups. Geographic range or stock area increased with abundance for each age group. Variation in abundance explained 63–94% of the variation in stock area. During periods of low abundance, the highest cod densities occurred in western regions of the southern Gulf for all age groups. Geographic distribution during these periods was more restricted for younger cod than for older cod. Density of age-3 cod was relatively high in only one of three western regions whereas densities of older cod were relatively high in two or all of these regions. As abundance increased, density increased slowly in the regions where cod were concentrated at low population sizes and more rapidly in surrounding regions. The greatest densities were found in the same area at low and high levels of abundance for younger cod but in different areas at these two abundance levels for older cod. We discuss this variation in geographic distribution in terms of density-dependent habitat selection and note its implications for stock assessment.

Nous avons vérifié les effets de la taille de la population sur la répartition géographique de la morue de l'Atlantique (*Gadus morhua*) dans le sud du golfe Saint-Laurent; nous avons étudié les groupes d'âges 3–8+ au cours de la période 1971–1990. La distribution dépendait de la densité pour les six groupes d'âge. L'aire de répartition des stocks a augmenté avec l'abondance pour chacun des groupes d'âge. La variation de l'abondance explique 63 à 94 % de la variation de l'aire de répartition des stocks. Durant les périodes de faible abondance, les plus fortes densités de morues ont été observées dans les régions occidentales du sud du golfe pour tous les groupes d'âge. Au cours de ces périodes, la répartition géographique est plus restreinte dans le cas des jeunes morues que dans celui des morues plus âgées. La densité des morues d'âge 3 était relativement élevée dans une seule des trois régions occidentales, tandis que la densité des morues plus âgées était relativement élevée dans deux ou dans l'ensemble de ces régions. Au fur et à mesure que l'abondance a augmenté, la densité a augmenté lentement dans les régions où la morue était concentrée lorsque la taille de la population était faible, et elle a augmenté rapidement dans les régions avoisinantes. Les densités les plus élevées ont été observées aux mêmes endroits, que l'abondance ait été faible ou élevée, dans le cas des jeunes morues, mais dans des endroits différentes dans celui des morues plus âgées. Nous traitons de cette variation de la répartition géographique de la morue en termes de sélection de l'habitat liée à la densité et soulignons les implications de cette constatation pour l'évaluation des stocks.

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Expansion or contraction of geographic range with increasing or decreasing population abundance has been observed in a variety of animals (reviewed by MacCall 1990). This observation has important implications for fisheries management. Most models used in fish stock assessment assume that catchability, the proportion of the population captured by a unit of effort, is a constant. However, Paloheimo and Dickie (1964) noted that catchability is inversely proportional to the area occupied by a stock. Thus, if abundance and geographic range are positively correlated, catchability should increase as population size decreases. Relationships among catchability, population size, and stock area or school size have been widely examined in pelagic fishes (reviewed by Winters and Wheeler 1985; MacCall 1990) but have received less attention in demersal fishes. An inverse relationship between catchability and population size has been reported for several demersal stocks (e.g., Pope and Garrod 1975; Houghton and Flatman 1980; Cook and Armstrong 1985; Crecco and Overholtz 1990; Gordoa and Hightower 1991; Rose and Leggett 1991), but a relationship between stock size and occupied range has been examined only rarely for these fishes, once at small scales (Rose and Leggett 1991) and once at large scales (Crecco and Overholtz 1990).

MacCall (1990) suggested that relationships between geographic range and population abundance may result from density-dependent habitat selection. Density-dependent habitat selection has been demonstrated in a variety of animals including fishes (reviewed by MacCall 1990 and Rosenzweig 1991). As density increases, selectivity decreases and habitat use expands to include suboptimal habitats. This decline in selectivity with increasing population size is predicted from the principles of optimal foraging and intraspecific competition (Fretwell and Lucas 1970). Given an "ideal free distribution" (i.e., all individuals are capable of choosing the most suitable habitat and are free to occupy it), individuals should assort themselves such that expected fitness is constant across the

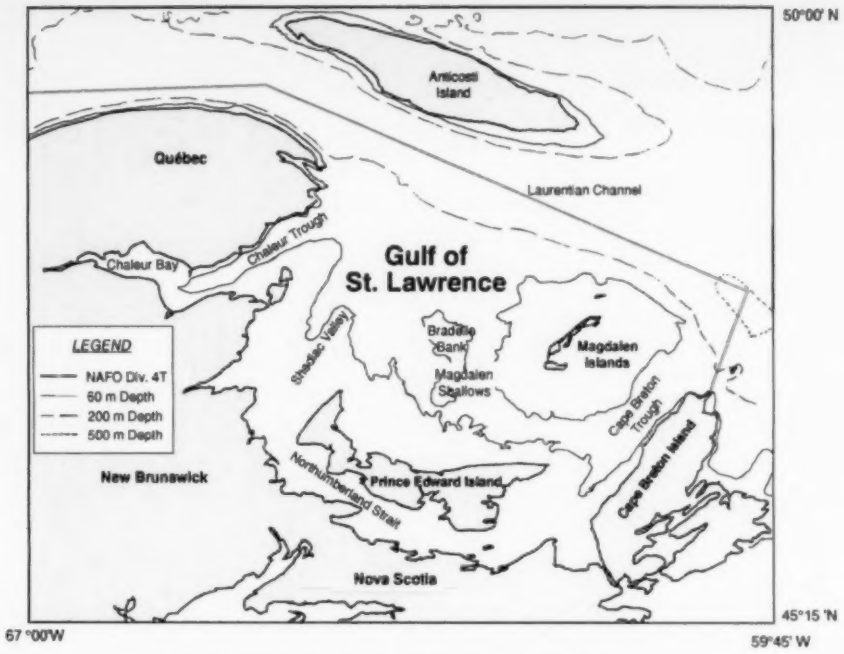

FIG. 1. Bathymetric map of the southern Gulf of St. Lawrence.

spectrum of occupied habitats. At low population sizes, individuals should occupy only the habitats with the highest basic suitability (where basic suitability is defined as the suitability when density approaches zero). As population size increases, density will increase in these preferred habitats and the fitness of individuals occupying them will decline due to intraspecific competition, until previously less suitable habitats become equally attractive and are occupied.

The theory of density-dependent habitat selection leads to several predictions about relationships among spatial pattern, habitat quality, and population abundance. If the ideal free distribution holds, then the geographic pattern of population density will "map" basic habitat suitability (MacCall 1990). However, deviations from a simple ideal free distribution are predicted when individuals differ in competitive abilities (e.g., Parker and Sutherland 1986) or when foraging rate is reduced by interference among competitors (e.g., Gillis and Kramer 1987). Gillis and Kramer (1987) found that such deviations were significant only at high densities and suggested that caution should be exercised when inferring patterns of habitat quality from animal distributions at high population sizes. Thus, the geographic pattern of basic habitat suitability may be best inferred from regional variation in density at low population sizes. Comparisons of the geographic patterns of density at low and high population sizes and of the relative rates of local density change with changes in population abundance can also provide information on processes underlying the density dependence of spatial pattern (e.g., Morris 1987, 1988).

In this study, we test effects of population size on the geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. The southern Gulf cod stock is well suited for these tests because (1) abundance of this population has varied widely over the last 20 yr and (2) 20-yr time series of both commercial catch rate data and fisheries-independent research survey data are available for this stock, allowing independent estimates of population abundance and geographic distribution. We examine two aspects of distribution. First, we test effects of abundance on geographic range of this population. Second, we test for regional variation in rates of local density change with changes in population abundance and compare regional variation in density between periods of low and high abundance.

Material and Methods

Study Area and Population

The southern Gulf of St. Lawrence (NAFO district 4T) comprises a broad, shallow (mostly <75 m) shelf, the Magdalen Shelf, bordered on the north by the Laurentian Channel, a deep, steep-sided trough with a maximum depth of 535 m (Fig. 1). Physical oceanography of the southern Gulf is described in Strain (1988). Two distinct water layers are present in winter: a surface mixed layer (50–100 m thick) with salinity between 31 and 32 ppt and temperatures near the freezing point (−1.5°C) and a deep, warm (4–6°C), saline (near 34.6 ppt) layer. In summer, three distinct water layers are present: a thin, warm, relatively fresh surface layer (10–30 m, 15°C, 27–32 ppt), an intermediate cold layer (−0.5–1°C, 31.5–33 ppt), and the deep warm layer. The intermediate cold layer covers the bottom of much of the central part of the southern Gulf on and around Bradelle Bank (Koeller 1981). Temperatures increase shoreward from this central area and with depth along the Laurentian Channel.

Since 1971, size of the southern Gulf cod population has varied between an estimated minimum of 131 million fish aged 3 and older in 1975 to an estimated maximum of 595 million in 1983 (Hanson et al. 1991). Estimated abundance was

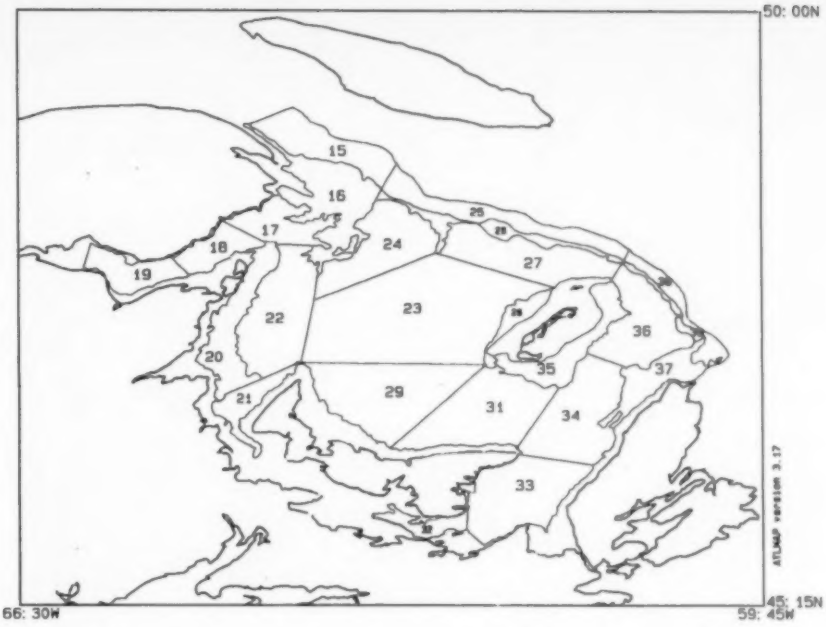

FIG. 2. Strata used in abundance surveys of the southern Gulf of St. Lawrence, September of 1971–90.

consistently low (<221 million 3+ fish) from 1971 to 1976 and consistently high (>450 million 3+ fish) from 1982 to 1990. This population exhibits an age-dependent annual migration: adult cod migrate between summer spawning and feeding grounds in the southern Gulf and overwintering grounds in the deeper water of the Sidney Bight (NAFO district 4Vn) whereas juvenile cod remain in the southern Gulf throughout the year (Jean 1964; Martin and Jean 1964). In summer and early fall, distribution within the southern Gulf is also age dependent, with older cod occurring at greater depths (Tremblay and Sinclair 1985; Swain 1993).

Geographic Distribution

We describe geographic distribution during September when cod are believed to have penetrated furthest into the Gulf and to be most widely distributed (Clay 1991). Spatial pattern was estimated each year from 1971 to 1990 from bottom trawl surveys conducted in the southern Gulf each September. Surveys used a stratified random design (Fig. 2), with stratification based on depth and geographic region. Station allocation was roughly proportional to stratum area. In addition to the randomly selected stations, 13 fixed stations were fished each year between 1971 and 1987. Surveys were conducted by the stern trawler *E.E. Prince* using a Yankee 36 trawl during the period 1971–85 and by the stern trawler *Lady Hammond* using a Western IIA trawl during the period 1986–90 (see Carrothers 1988 for detailed trawl specifications). A comparative fishing experiment in 1985 failed to reveal significant differences in catch rates for cod between the two vessels and gears (G. Nielsen, unpublished data). Fishing by the *E.E. Prince* was during daylight hours only, while the *Lady Hammond* fished 24 h per day. However, a comparative fishing experiment in 1988 revealed no significant differences between day and night catches of cod (G. Nielsen, unpublished data).

The target fishing procedure was a 30-min tow at 3.5 knots in all years. All catches were adjusted to a standard tow of 1.75 nautical miles. Cod numbers and length frequency distributions were determined for each catch. Otoliths were removed from a length-stratified subsample of each catch for age determination. Sample sizes for each year ranged from 61 to 164 tows. Spatial coverage of the survey area was complete in all years except 1978 (strata 24, 25, and 28 without coverage), 1983 (stratum 21 without coverage), and 1988 (stratum 21 without coverage).

Because spatial pattern is age dependent in this population (Tremblay and Sinclair 1985; Swain 1993), we estimated geographic distribution separately for each age between 3 and 7 and for fish aged 8 and older. In each year, age composition was estimated for each tow by applying a survey-wide age–length key to the length distribution of the catch of each tow using the RVAN procedure MALKEY (Clay 1989).

We calculated an index of the relative area occupied by each age group in each year by estimating the area over which fish density (number per standard tow) exceeded a specified level. For each age group, this level was the 60th percentile of the catch per tow of that age group over the 20-yr time series (1685 tows). This procedure is similar to that used by Crecco and Overholtz (1990), except that they used the 50th percentile. We chose the 60th percentile because cod density never reached higher levels (i.e., the 70th percentile) for some age groups in years of low abundance whereas density exceeded lower levels (i.e., the 50th percentile) over the entire survey area in years of high abundance. The 60th percentile level was 9, 14, 15, 11, 6, or 8 fish per tow for ages 3–7 or 8+, respectively. Area was calculated by mapping the 60th percentile contour within

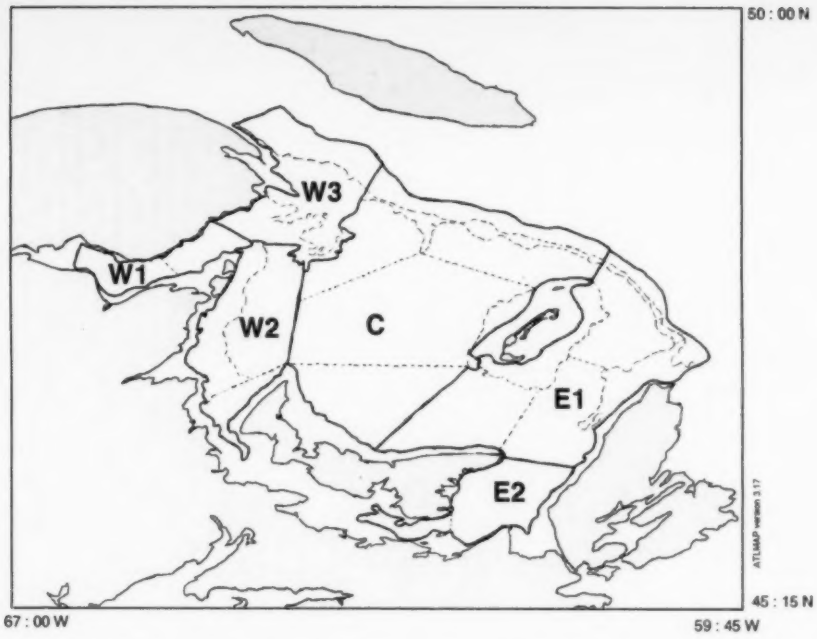

FIG. 3. Grouping of southern Gulf of St. Lawrence strata into six broad geographic regions.

the survey area for each of the 120 age–year combinations. Mapping was by ordinary kriging (Matheron 1971; Journel and Huijbregts 1978). This geostatistical technique uses the spatial covariation among samples to estimate density at points on a fine-mesh grid used for mapping. The technique corrects for spatial variation in sampling intensity by assigning lower weights to closely set sample points. (See Simard et al. 1992 for an evaluation of geostatistical methods in marine ecology.) Stratum 32 and part of stratum 33 were inadvertantly excluded from this analysis, but their exclusion had little effect on stock area calculations because cod were usually rare in these areas.

For each age group, the effect of population size (N) on stock area (A) was tested by nonlinear least squares regression analysis using the NLIN procedure of SAS (SAS Institute 1990). Because stock area could not exceed a maximum value of 67 870 km^2 (the total survey area included in the kriging analysis), we used an asymptotic regression model:

$$(1) \quad A = \beta_0 - \beta_1(\exp(-\beta_2 N)).$$

Plots of residuals from the fitted models confirmed that models of this form were appropriate for the data. Because spatial distribution is age dependent in cod, spatial pattern of a particular age-class might be expected to depend most strongly on population size of that age-class. However, because spatial distribution is overlapping among age-classes, spatial pattern may be affected by competition among as well as within age-classes. Thus, we performed two tests with this asymptotic model. First, we related A_{ij}, the area occupied by age group i in year j, to N_{ij}, the population size of age group i in year j. Second, we related A_{ij} to the total population size of fish aged 3 and older.

Strata were grouped into six broad regions (Fig. 3) to determine whether density increased uniformly in all areas of the southern Gulf as population size increased. Homogeneity of the slope of catch rate (fish per standard tow) on population abundance was tested among regions for each age group using a linear model with ln(catch rate + 0.001) as the dependent variable, population abundance of the age group as a covariate, and region and stratum nested within region as fixed effects.

To display changes in the relative distribution of cod among regions between periods of low and high abundance, we calculated the stratified mean catch rate in each region for each of the 5 yr of lowest abundance and each of the 5 yr of highest abundance for each age group. We plotted the mean regional catch rates for each of the two periods, scaled by the maximum catch rate in each period to emphasize any period × region interaction. We tested this interaction using a linear model with ln(catch rate + 0.001) as the dependent variable, region, stratum nested within region, abundance period, and the region × abundance period interaction as fixed effects, and year nested within abundance period as a random effect. This and the previous analysis used the GLM and REG procedures of SAS.

Population Size

Sequential population analysis (SPA) was used to estimate midyear population sizes. Annual assessments of this stock use SPA calibrated with both research survey and commercial catch rate indices (e.g., Hanson et al. 1991). We needed independent estimates of spatial pattern and population size to permit statistical tests of significance (cf. Crecco and Overholtz 1990; Gordoa and Hightower 1991). Because the research survey data were used to estimate spatial pattern, we used a SPA calibrated with the commercial catch rate index only. This index was for otter trawls with effort standardized following Gavaris (1980). Details of the effort standardization and the standardized catch rates at age for 1971–90 are given in Hanson et al. (1991). For a variety of reasons (e.g., variable catchability), commercial

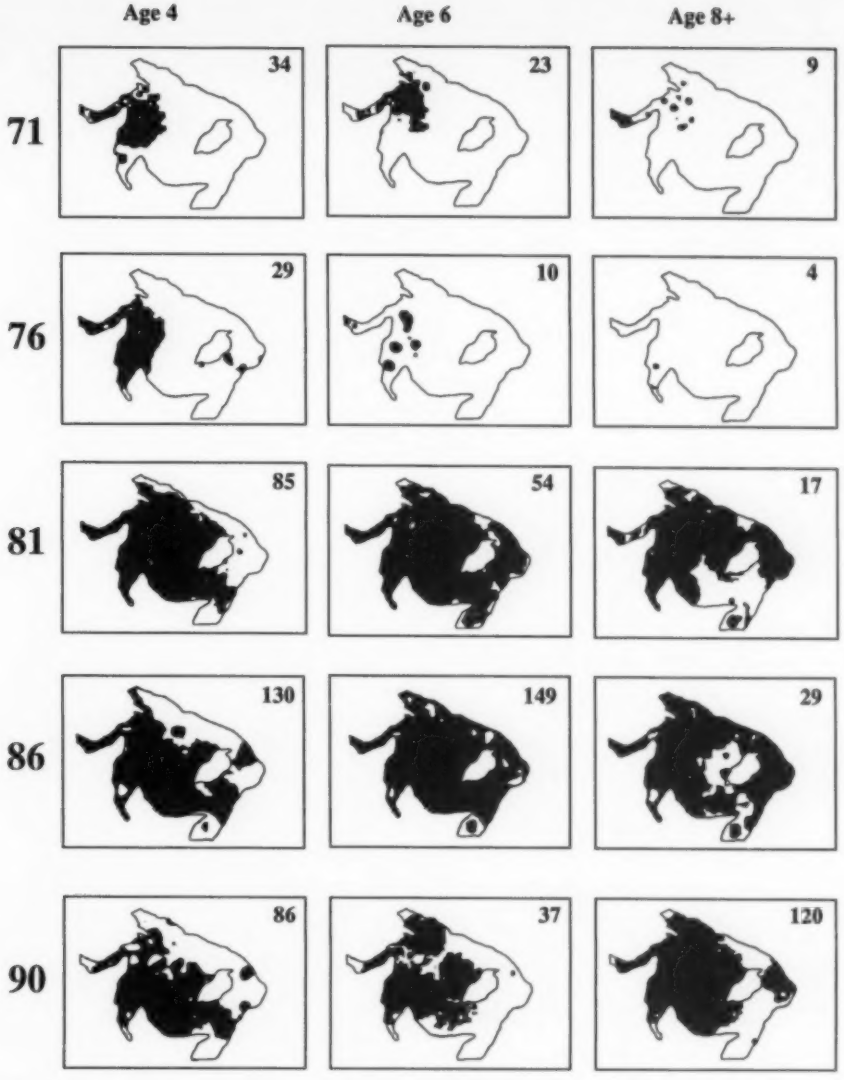

FIG. 4. Stock areas of cod in the southern Gulf of St. Lawrence in September for selected ages and years. Numbers inside panels are total abundance of the age group in millions of fish.

catch rates may not provide reliable estimates of relative abundance (e.g., Pope and Garrod 1975; Winters and Wheeler 1985; Crecco and Overholtz 1990). However, for the southern Gulf cod stock, similar trends are shown by both the research survey and the commercial otter trawl catch rate indices, and population sizes estimated by the SPA calibrated with the commercial catch rates only are closely correlated with those estimated by calibrating with both catch rate indices ($R^2 > 0.9$).

Results

Examples of the variation in stock area among age groups and years are shown in Fig. 4. Cod were concentrated in western regions of the southern Gulf at low population sizes and expanded to the east as population size increased. Stock area depended on population size for all six age groups (Fig. 5; $P = 0.0002$ for age 3, $P < 0.0001$ for ages 4–8+). As abundance increased, stock area increased toward an asymptote near or below the total survey area (Table 1). The increase in stock area with increasing population size was progressively steeper for older ages. Variation in abundance explained 63–94% of the variation in stock area (Fig. 5). The better predictor of the stock area of a particular age group was population size of that age group (Table 2). Total population size of fish aged 3 and older was a substantially poorer predictor of age-specific stock area for ages 5–8+. However, relationships between stock area and both population size variables were highly significant ($P < 0.001$).

Density did not increase uniformly over all regions of the southern Gulf as population size increased. Differences among

TABLE 1. Parameter estimates for the asymptotic relationships between stock area (hundreds of square kilometres) and abundance (millions of fish) for age-classes 3–8+ of cod in the southern Gulf of St. Lawrence. Parameters are from equation 1 in the text. F is the ratio of explained to residual MS and P is the associated significance level (df = 2,17).

Age	β_0	β_1	β_2	F	P
3	576.95	636.93	0.01267	14.572	0.0002
4	535.37	762.87	0.02157	30.707	<0.0001
5	613.76	804.27	0.02073	58.271	<0.0001
6	676.89	860.93	0.02857	128.616	<0.0001
7	667.56	837.48	0.05429	127.293	<0.0001
8	606.88	870.92	0.07232	73.864	<0.0001

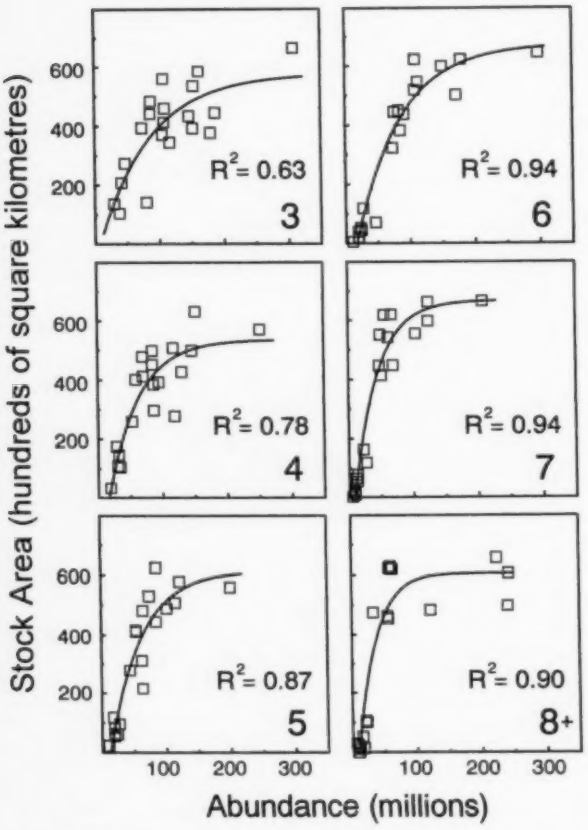

FIG. 5. Relationship between age-specific stock area and abundance for age groups 3–8+. The line is the predicted relationship given the model in equation 1. Parameter values are given in Table 1.

regions in the slope of catch rate on population size were highly significant ($P < 0.0001$) for all ages (Fig. 6). For ages 3 and 4, slopes were highest for regions W3 and C and lowest for W2. For ages 5–8+, slopes were highest for regions E1 and C and low for regions W1, W2, and W3. Slope tended to be low and nonsignificant in region E2 for all ages.

During periods of low abundance, cod densities were greatest in region W2 (age 3) or W1 and W2 (ages 4–8+; Fig. 7). Density in region W3 during periods of low abundance was relatively low for younger cod (ages 3 and 4) but relatively high for older cod (especially ages 6 and 7). Thus, slopes of catch rate on abundance tended to be lowest in the regions with the highest concentrations of cod during periods of low abundance, and in region E2 where cod were rare at all population sizes.

TABLE 2. Coefficients of determination (R^2) for models relating age-specific stock area to abundance. Models use either of two abundance variables: N_i, the abundance of age-class i, and N_T, the total abundance of fish aged 3 and over.

Age	N_i	N_T
3	0.63	0.57
4	0.78	0.78
5	0.87	0.72
6	0.94	0.68
7	0.94	0.67
8	0.90	0.65

The interaction between abundance period and the effect of region on catch rates (Fig. 7) was highly significant ($P < 0.0001$) for all ages. Cod distribution expanded with increasing abundance for all age groups. At low population sizes, cod were concentrated in western regions of the southern Gulf. At high population sizes, a higher proportion of cod occurred outside the "preferred" western region(s). This expansion of spatial distribution was more striking for the older age groups. For age 3, cod density was highest in region W2 during both abundance periods. For ages 4–8+, peak cod density shifted from regions W1 and/or W2 to region W3, or W3 and C. Density of ages 5 and 6 in region C, only 22 or 27% of the peak density in W2 or W1 at low abundance, exceeded the density in W2 or W1 at high abundance (161 and 206%, respectively). Density in region E1 was only 9% (age 3) to 27% (age 8+) of the peak density in W1 or W2 at low abundance but approached or exceeded density in these regions at high abundance for ages 5–8+ (80–88% for ages 5, 7, and 8+; 115% for age 6).

Discussion

This study demonstrates that the area occupied by cod in the southern Gulf of St. Lawrence increases as cod abundance increases. This result conforms to the widely predicted relationship between stock area and abundance (e.g., Paloheimo and Dickie 1964; Pope and Garrod 1975) and agrees with results for haddock (*Melanogrammus aeglefinus*) on Georges Bank (Crecco and Overholtz 1990), for cod at small scales at Brador Bay (Rose and Leggett 1991), and for a number of pelagic fishes (e.g., Winters and Wheeler 1985; MacCall 1990).

The geographic pattern of density at low population sizes suggests that the optimum habitat for cod of all ages (3–8+) tends to be concentrated in western regions of the southern Gulf, in particular regions W2, the Shediac Valley – Miramichi Bay

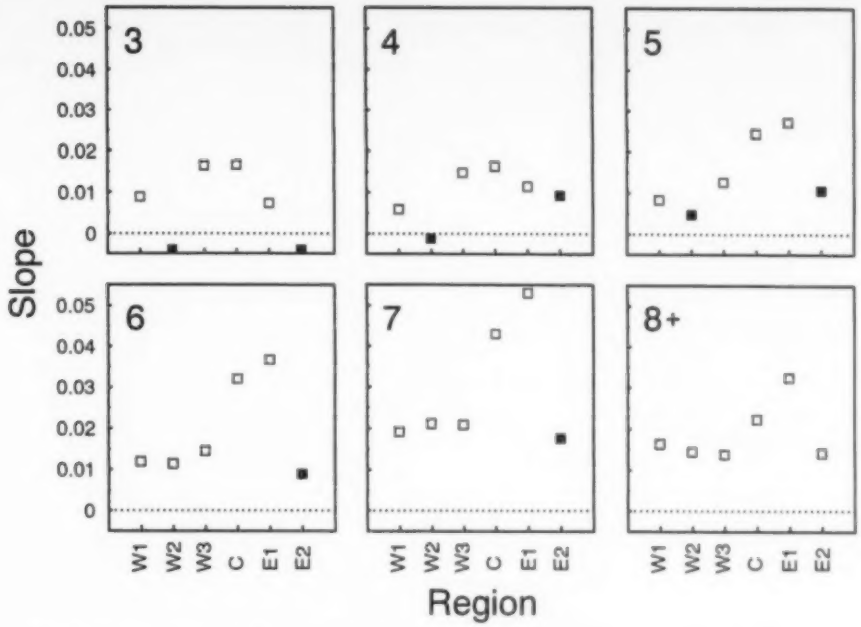

FIG. 6. Slopes of log-transformed regional catch rate (fish per standard tow) versus population size (millions of fish) for age groups 3–8+. Solid squares do not differ significantly ($P > 0.05$) from zero.

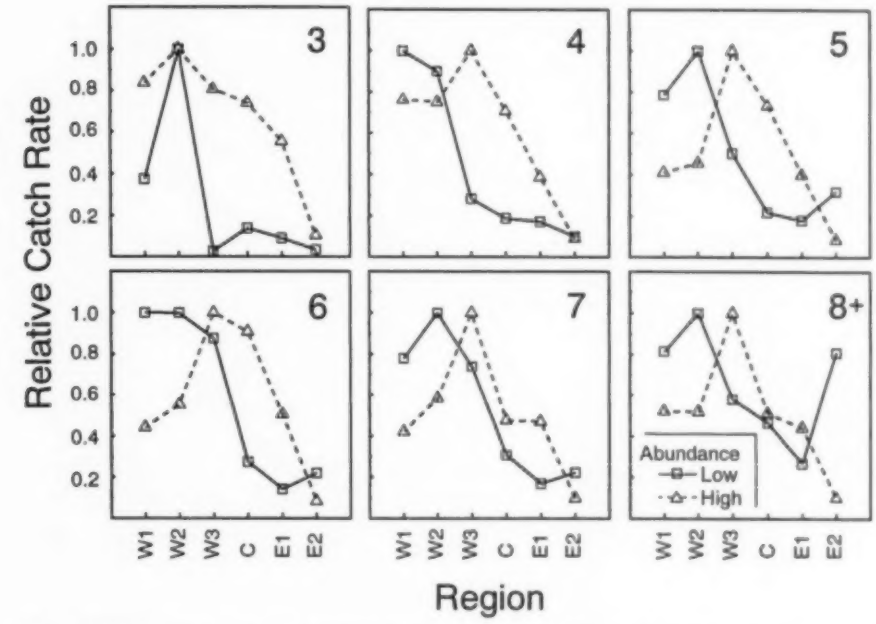

FIG. 7. Mean regional catch rates for the 5 yr of lowest abundance (squares, solid line) and the 5 yr of highest abundance (triangles, broken line) for age groups 3–8+. Catch rates are scaled by the maximum mean catch rate within each abundance period.

area, and W1, Chaleur Bay. The factors that determine habitat quality for cod are not well known. Cod distribution is related to environmental parameters such as depth, temperature, salinity, and bottom type (e.g., Scott 1982; Mahon et al. 1984; Tremblay and Sinclair 1985; Perry et al. 1988; Smith 1990; Smith et al. 1991; Sinclair 1992; Swain 1993). However, it is unlikely that geophysical factors alone determine cod distribution (e.g., Rose and Leggett 1989). Rose and Leggett (1989) found that the mesoscale spatial pattern of cod in Brador Bay was explained by interactive effects of water temperature and

prey density. The density of prey species is likely to similarly be an important determinant of habitat quality for cod in the southern Gulf of St. Lawrence.

As population size increased, density increased slowly in the regions where cod concentrations were highest at low population sizes (W2, W1) and more rapidly in surrounding regions (W3, C, E1). For cod older than age 3, the area of highest densities actually shifted from the regions preferred at low abundance to surrounding regions at high abundance. Perhaps this spatial shift in peak densities of older cod reflects factors unrelated to population size but coincidently confounded with it. Such factors could include a shift in the spatial pattern of basic habitat suitability in recent years of high abundance, or earlier migration of older cod out of the southern Gulf in years of high abundance.

Alternatively, this shift in peak density might be explained by density-dependent habitat use. One possibility is that per capita reductions in foraging efficiency due to interference competition are more severe in the "better" habitats (i.e., the habitats with higher basic habitat suitability), leading to higher densities in the "poorer" habitats at high population sizes (e.g., see fig. 2D in Morris 1988). This could occur if the more aggressive individuals occupied the better habitats, as is predicted when there is interference (Parker and Sutherland 1986). Brawn (1961) observed aggressive behaviour in North Sea cod during the fall feeding period and suggested that this behaviour may represent a spacing mechanism that disperses cod more widely over the feeding grounds. However, aggressive forms of interference may be of limited importance at high population densities (e.g., Gillis and Kramer 1987), and it is difficult to imagine that agonistic interactions could constrain spatial pattern on the large scales involved in this study.

A second possibility, suggested to us by D.L. Kramer (McGill University, Montreal, Que., personal communication), involves an interaction between effects of a density-dependent resource (e.g., food, whose benefit per individual declines with increasing numbers of individuals) and a density-independent factor (e.g., water temperature, whose costs or benefits do not depend on population size). When density-independent costs are greater in the habitats with higher levels of density-dependent resources, then densities are predicted to be highest at low population sizes in the habitats with the highest resource levels and at high population sizes in the habitats with the lowest costs (D.L. Kramer, personal communication). Thus, the shift in peak densities from regions W1 and W2 at low population sizes to W3 and C at high population sizes might involve a shift from high-resource, high-cost areas to low-resource, low-cost areas. Variation among these areas in bottom temperature, an important determinant of metabolic rate in fishes (e.g., Fry 1971), is consistent with this possibility. Bottom temperatures tend to be relatively warm in regions W1 and W2, particularly W2, and relatively cold in regions W3 and C, particularly C (D.P. Swain, unpublished data). Because metabolic costs are lower at colder temperatures (Fry 1971), regions W1 and W2 are "high-cost" areas whereas regions W3 and C are "low-cost" areas.

Spatial distribution appeared to be more restricted for younger cod than for older cod. Younger cod were less widely distributed at low population sizes (Fig. 7), stock area expanded more slowly for younger cod as their abundance increased (Fig. 5), and shifts in relative density among regions were smaller for younger cod between low- and high-abundance periods (Fig. 7). These results might be explained by less severe competitive interference among younger than among older cod or by wider variation in habitat quality for younger than for older cod. Habitat quality may vary less widely for older cod if diet is less restricted for older individuals (e.g., Helfman 1978) or if greater mobility and metabolic efficiency permit older individuals to exploit less productive habitats than younger individuals (Haedrich and Rowe 1977). If so, the better habitats will lose their fitness advantage over lower quality habitats sooner for older cod, and their spatial pattern should expand more rapidly as density increases.

Competitive ability usually depends on size, with larger individuals at advantage (e.g., Henderson and Chiszar 1977). However, density-dependent changes in spatial pattern were greater for older cod than for younger cod. This suggests that interaction between age-classes of cod is limited. Otherwise, we expect that younger cod would be displaced by older cod and that regional shifts in peak density as abundance increases would be greater for younger cod rather than for older cod. Diet is size dependent in cod (e.g., Kohler and Fitzgerald 1969; Daan 1973; Langton 1982; Durbin et al. 1983; Rice 1988). Thus, competitive (and agonistic) interaction may be most intense among similarly sized individuals. Interaction between age-classes may also be limited by small-scale variation in habitat selection as a result of differences in diet (e.g., dos Santos and Falk-Petersen 1989).

Fitness should decline as density in the optimum habitat increases and habitat use expands to include suboptimal habitats. We have no information on most natural components of fitness in this population (e.g., natural mortality rates). Growth rates do conform to this prediction, declining sharply at high population sizes in this stock (Hanson and Chouinard 1992). However, factors other than competition and declining habitat quality (e.g., size-selective fishing mortality) may also contribute to the decline in growth rate in recent years of high abundance in this population (Hanson and Chouinard 1992).

Density-dependent spatial pattern may have important implications for the management of this stock. Catchability is predicted to be inversely related to the area occupied by a stock (Paloheimo and Dickie 1964), yet it is assumed to be constant in stock assessment calculations. Results of this study suggest that catchability of cod in the southern Gulf should increase as population size decreases. This possibility is currently under investigation.

Acknowledgements

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Partial Recruitment Considerations in Setting Catch Quotas

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An approach is presented for investigating the interactions between fishing fleets that compete by exploiting different age-groups of the same resource population. The term "partial recruitment" (PR) is used to describe the age-specific exploitation pattern experienced by a population, either from individual fishing fleets or the combined effects of several fleets. Methods are presented to calculate catch quotas for the individual fleets if the management objective is to keep fleet effort constant, or alternatively to predict catch rates by fleet if the allocation rules are based on a percentage sharing of the total allowable catch (TAC) among fleets. Simulations based on an Atlantic cod (*Gadus morhua*) fishery on the Nova Scotian Shelf is used to illustrate the method. The results indicate the importance of considering differences in PR among competing fishing fleets when setting catch quotas. The relative effort exerted by the fleets will affect target fishing mortalities. The fleet that concentrates on younger fish can intercept recruitment. Since the fleets exploit different age-groups, changes in fishable biomass due to recruitment variation are lagged, and fishing success will vary among fleets. Understanding the dynamics of PR may help explain why certain fleets either exceed or fall short of catch quotas.

On présente une approche pour étudier les interactions entre les flottes de pêche qui se font concurrence en exploitant des groupes d'âge différents au sein d'une même population d'une ressource halieutique particulière. L'expression « recrutement partiel » (RP) est utilisée pour décrire le type d'exploitation spécifique de l'âge que connaît une population, soit par l'action de flottes de pêche individuelles soit par l'action combinée de plusieurs flottes. On décrit des méthodes pour calculer les contingents de capture pour les flottes individuelles si l'objectif de gestion est de garder l'effort de la flotte constant, ou encore, pour prévoir les taux de prises par flotte si les règles d'attribution des contingents sont fondées sur un partage (en pourcentage) du TPA entre les flottes. Des simulations portant sur la pêche à la morue franche (*Gadus morhua*) sur le plateau de la Nouvelle-Écosse sont utilisées pour illustrer la méthode. Les résultats indiquent l'importance de prendre en considération les différences de RP entre les flottes concurrentes lorsqu'on établit les contingents de capture. L'effort relatif exercé par les flottes influe sur les mortalités par pêche cibles. La flotte qui concentre ses efforts sur le poisson plus jeune peut intercepter le recrutement. Comme les flottes exploitent des groupes d'âge différents, les changements de la biomasse exploitable attribuables à la variation de recrutement sont déphasés, et le succès de la pêche variera d'une flotte à l'autre. Comprendre la dynamique du RP pourrait aider à expliquer pourquoi certaines flottes dépassent leur contingent de capture ou ne l'atteignent pas.

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Stock-specific total allowable catches (TACs) and fleet-specific catch allocations (quotas) are widely used to manage groundfish fisheries on the Canadian east coast (Anonymous 1992). The management strategy is to maintain fishing mortality at the $F_{0.1}$ target exploitation level (Gulland and Boerema 1973). Where suitable databases exist, the TACs are based on catch projections 1 or 2 yr into the future using population-at-age estimates for the current year, average weights-at-age, the target fishing mortality, and a fishery-wide age-specific exploitation pattern, referred to here as partial recruitment (PR). Once a TAC is set, allocations are made to fleets as part of a policy to distribute access to the resource equitably. Fleets are defined by the type of fishing gear used and vessel size. There are two broadly defined fleet sectors in eastern Canadian groundfish fisheries, the fixed gear sector which is composed of longlines, traps, gill nets, and handlines, and the mobile component which includes otter trawls and Danish and Scottish seines. The basis for the allocations has commonly been a recent average proportion of yield (weight) by fleet.

Provisions exist for the transfer of quotas among fleets during a fishing year if it appears that one fleet is unlikely to catch its quota. It has been observed in some areas that the mobile fleets have caught their quotas quickly, while the fixed gear sectors have had shortfalls. This has led to several midseason quota transfers to accommodate the needs of the mobile gear fleets. This paper presents a methodology for investigating interactions among gears when original allocations or midseason reallocations are made.

This paper presents an approach for examining interactions among fleet sectors which are caused by differences in PR in the context of the quota allocation regime used in eastern Canada. I have developed an approach for estimating fleet-specific and fishery-wide PR resulting from different combinations of fleet fishing effort. The long-term yield per recruit implications of these mixed gear fisheries are analyzed. Methods for investigating the short-term interference of one fleet on another are also presented. Finally, the effect of recruitment variability on the catch allocation regime is described. A case study is presented for the Northwest Atlantic Fisheries

TABLE 1. Summary of abbreviations. For the most part, these follow the conventions used in Ricker (1975).

$\bar{B}$	Fishable biomass, the biomass of the population that is vulnerable to fishing, calculated as the product of mean population numbers, weight at age, and PR
C	Catch numbers
E	Fishing effort
F	Instantaneous rate of fishing mortality
i	Index of age
j	Index of year
k	Index of fishing fleet
M	Instantaneous rate of natural mortality
N	Population numbers at the beginning of the time period
$\bar{N}$	Average population numbers in the time period, calculated according to equation 1.15 of Ricker (1975)
q	Catchability coefficient
S	Partial recruitment
TAC	Total allowable catch
W	Weight-at-age
Y	Fishing yield in weight
Z	Instantaneous rate of natural mortality, the sum of F and M

Organization (NAFO) Div. 4VsW Atlantic cod (*Gadus morhua*) fishery where two gears are dominant, otter trawls and longlines. In this case, otter trawls catch younger fish than longlines. These methods have general application to multigear fisheries for which age-structured estimates of population and fishing mortality are available. They may be useful when making resource allocation decisions.

Methods

PR is a term commonly used in eastern Canadian stock assessments to describe the proportion of an age-group in a population that is vulnerable to fishing. Ages not fished have a PR of 0, and the age(s) with the highest fishing mortality are called fully recruited and have a PR of 1. It may apply to the total fishery or to a specific fishing fleet. Two factors determine the PR of an age-group to a specific fleet, the selectivity of the gear used as determined by its characteristics (e.g., mesh size or hook size) and the availability of the age-group to the gear (Beverton and Holt 1957, section 8.1). The latter may be influenced by age segregation in populations and the grounds fished by the fleet (Sinclair 1992). The PR of the total fishery depends on the relative fishing effort by the fleets involved. The part of the population that is vulnerable to fishing is calculated as the product of the PR and the population at age vectors. I refer to the vulnerable population as the fishable population (i.e., that within the selected size range and on the fishing grounds).

This concept of a fishable population is important in interpreting basic fisheries data. Classic theory relates catch in numbers (C), fishing effort (E), and mean stock size ($\bar{N}$) during a fishing season as

$$(1) \qquad \frac{C}{E} = q\bar{N}$$

where q = the catchability coefficient (Table 1). Ricker (1975) defined catchability as the fraction of a fish stock that is caught by a unit of fishing effort. This definition would be more appropriate if "fishable" stock were used. If not, q would vary as the relative size of the unrecruited portion of the stock varies. Catch per unit effort (CPUE) reflects trends in the fishable population and not the total population. A more complete equation would include PR at age (S_i) as

$$(2) \qquad \frac{C}{E} = q\sum_i S_i \bar{N}_i .$$

Similarly, the relation between effort and fishing mortality (F) is mediated through q. F is taken to mean the fishing mortality on fully recruited ages:

$$(3) \qquad F = qE.$$

Substituting for E in equation 2, we see that the ratio C/F will provide an estimate of the fishable population numbers. Yield in weight divided by F will be the mean fishable biomass ($\bar{B}$).

Partial Recruitment

PR by gear sector was estimated in the following manner. Estimates of F at age (i) in a given year (j) from cohort analysis (Pope 1972) were partitioned among gears (k) using the gear specific catch at age:

$$(4) \qquad F_{ijk} = \frac{F_{ij} C_{ijk}}{\sum_k C_{ijk}} .$$

Assuming that age and year effects are separable (Pope and Shepherd 1982), age and year effects were estimated using dummy variables in a multiple regression (Draper and Smith 1966) using the model

$$(5) \qquad \ln F_{ijk} = \beta_0 + \beta_1 I + \beta_2 J + \varepsilon$$

where β_0 = the reference level for a given age and year, β_1 = a vector of age effects, I = a matrix of 0 and 1 designating the ages associated with F_{ijk}, β_2 = a vector of year effects, and J = a matrix of 0 and 1 designating the years associated with F_{ijk}. The age effects, standardized to the highest value, were interpreted as the PR for each gear sector.

Fishing mortalities and catch-at-age were taken from Sinclair and Annand (1986). Two gears were used, longlines and otter trawls. The years 1982–84 and ages 3–10 were used. Insufficient sampling of ages greater than 10 led to large variations in estimated PR. Consequently, the estimated values for age 10 were applied to ages 11–15 in further analyses.

Given these estimates of PR for the two gears, fishery-wide PRs were computed for hypothetical two-gear fisheries with different proportions of longline effort. Assuming that the two gears had the same fishing power (generate the same fully recruited fishing mortality per unit effort) and fish the same stock simultaneously with effort proportions equal to δ_k, then the resulting fishery-wide PRs (S_i) were calculated in two steps:

$$(6) \qquad Q_i = \sum_k S_{ik}\,\delta_k$$

$$(7) \qquad S_i = \frac{Q_i}{\max\,[Q_i]}$$

where the denominator in equation 7 is the maximum value of Q_i across all ages. Equation 7 is a necessary standardization if both gears do not have full recruitment at the same age.

Yield per Recruit

No differences in weights at age (W_i) were found between gears in the 4VsW cod fishery, and thus, these were held con-

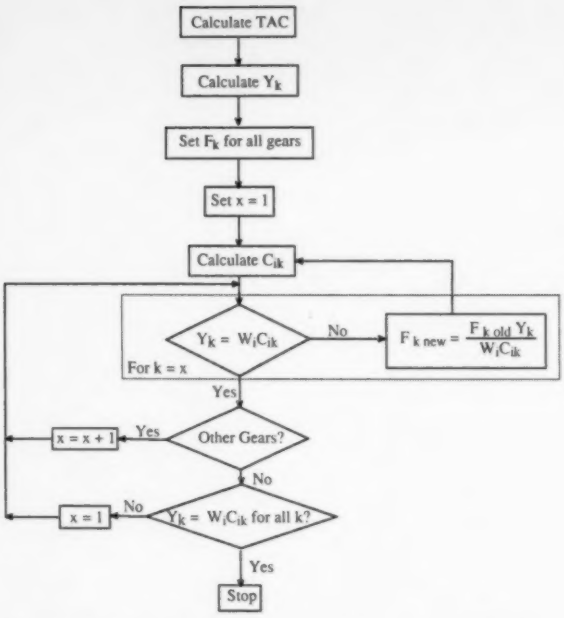

FIG. 1. Flow chart for calculating gear-specific catch quotas under the fixed percentage yield allocation regime. Gear-specific fishing mortalities (F_k) which satisfy the yield constraints (Y_k) were found with an iterative procedure described in the Methods section.

stant. The commercial weights at age for 1985 were used in the case study (Sinclair and Annand 1986). Natural mortality (M) was assumed to be 0.2, consistent with the value used in most cod assessments.

A series of Thompson and Bell yield per recruit analyses (Rivard 1982) were run using the fishery-wide PR patterns calculated for the simulated two-gear fisheries. Comparisons were made with respect to $F_{0.1}$, F_{max}, yield per recruit, gear-specific fully recruited fishing mortality, and CPUE for each level of longline participation. Gear-specific fishing mortalities for any level of fishery-wide fishing mortality (F) were calculated as

$$(8) \qquad F_k = \frac{\delta_k F}{\max [Q_i]}$$

where i = the age of highest fishing mortality in the fishery. For simplicity, standardized fishing effort (E_k) was expressed in the same units as the gear-specific fully recruited fishing mortality (F_k) (i.e., $q = 1$). The total standardized fishing effort is thus the sum of the gear-specific Fs. However, the population fully recruited fishing mortality (F) in a mixed fishery would be less than the total standardized fishing effort, since the two gears do not have full recruitment at the same age. The ratio between F and the total standardized effort is equal to max $[Q_i]$. If empirical estimates of catchability were available, one could calculate catch per nominal fishing effort.

Gear-specific yields and fishable biomasses at $F_{0.1}$ were calculated for simulated two-gear fisheries with different proportions of longline effort. Results are given on a per recruit basis. PR was taken from equation 7. The function MPROJECT from Rivard (1982) was used for the calculations. Yearly catch weight by gear (Y_k) was calculated as

$$(9) \qquad Y_k = \sum_i \left(\frac{W_i C_i S_{ik} F_k}{\sum_k S_{ik} F_k} \right)$$

and fishable biomass by gear as

$$(10) \qquad \bar{B}_k = \sum_i \bar{B}_i S_{ik}$$

where $\bar{B}_i$ = the mean population biomass at age i.

Interference

Response surfaces of yield per recruit as a function of various levels of F by the two gears were calculated using the Baranov catch equation and assuming that both gears fished the stock simultaneously. Catch-at-age by gear (C_{ik}) and the yield to each gear sector (Y_k) were calculated as

$$(11) \qquad C_{ik} = N_i \frac{(1 - e^{-(M + \sum_i S_{ik} F_k)}) S_{ik} F_k}{M + \sum_k S_{ik} F_k}$$

$$(12) \qquad Y_k = \sum_i C_{ik} W_i .$$

Recruitment Variability and Catch Allocation

The effect of recruitment variation on resource sharing and fleet fishing effort was investigated for two catch allocation scenarios. In the first, the catch was allocated to the gears based on a predetermined yield sharing rule. This is similar to the current system for eastern Canadian groundfish fisheries. In the second, catch was allocated on the basis of fixed effort per gear.

Simulations under the fixed percentage yield allocation regime were carried out as follows (see Fig. 1). Starting with a beginning-of-year population at age (N_i), the yearly TAC was calculated as

$$(13) \qquad \text{TAC} = \sum_i \frac{W_i N_i (1 - e^{-Z}) F_i}{Z_i}$$

where

$$F_i = F S_i$$

$$Z_i = F_i + M.$$

The initial values of S_i were those which resulted from a two-gear fishery at $F_{0.1}$ with yield proportions at the predetermined level ((50%:50%) in this example). The target fully recruited fishing mortality for the population (F) was the $F_{0.1}$ that corresponded to the S_i used to calculate the TAC. In subsequent years, PR was calculated as the average of the previous 3 yr, based on estimates of fishing mortality. In one year,

$$(14) \qquad S_i = \frac{F_i}{\max [F_i]} .$$

This is consistent with current stock assessment procedures. The TAC was then split between gears to give target quotas (Y_k). I used an iterative approach to find F_k in equation 11 which satisfied the yield constraint (equation 12). This consisted of choosing the same F for both gears and then calculating the catch-at-age and yield for one gear. The yield was compared with the target quota. The gear-specific F was adjusted by the

TABLE 2. PR estimates for simulated fisheries with different percentages of longline effort. The percent otter trawl effort is 100% minus the percent longline effort. Full recruitment to longline gear was at age 9–10. Full recruitment to otter trawls was at age 6–7 and was dome-shaped, declining at older ages. The age of full recruitment and the shape of the population PR curve depended on the mix of effort in two-gear fisheries.

	% longline effort					
Age	100% LL	80% LL	60% LL	40% LL	20% LL	0% LL
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.02	0.04	0.07	0.08	0.09
4	0.04	0.15	0.27	0.40	0.46	0.51
5	0.13	0.31	0.51	0.73	0.81	0.88
6	0.27	0.45	0.66	0.87	0.93	0.98
7	0.49	0.65	0.82	1.00	1.00	1.00
8	0.81	0.87	0.93	0.96	0.84	0.74
9	0.99	1.00	1.00	0.96	0.77	0.62
10	1.00	0.98	0.94	0.86	0.64	0.47
11	1.00	0.97	0.93	0.84	0.62	0.45
12	1.00	0.97	0.93	0.84	0.62	0.45
max[Q_i]	1.00	0.92	0.84	0.80	0.90	1.00

ratio of the target yield to the estimated yield. The process was repeated until the correct F for that gear was found. While holding this F constant, F for the second gear was adjusted to satisfy the respective quota. If the estimated yields differed from the targets, the procedure was repeated. The population for the next year ($t + 1$) was calculated as

$$N_{i+1,\,t+1} = N_{it}\,e_i^{-Z_i}. \quad (15)$$

The TAC for the next year was then calculated and the process repeated.

For the fixed F allocation option, yields by gear were calculated directly using equations 11 and 12. The gear-specific fishing mortalities used in this example were those which would result in a long-term 50%:50% yield sharing at $F_{0.1}$ with stable recruitment. The population in year $t + 1$ was calculated with equation 15.

For all of these simulations, recruitment estimates at age 3 were taken from Gagné et al. (1984) for the years 1958–69 and for 1970–86 from Sinclair and Smith (1987). Starting population size was taken as a stable age distribution resulting from fishing at $F_{0.1}$ for a PR from a fishery with equal otter trawl and longline effort and with average recruitment for the time period.

Results

Partial Recruitment

The PR pattern for otter trawls was dome-shaped with full recruitment at ages 6–7 (Table 2). PR then dropped to 45% by age 11. This is probably due to the concentration of otter trawling on the shallow banks. These cod tend to move to deeper water at older ages (Sinclair 1992), and thus the older fish are not as heavily exploited by the gear as the midaged fish. Longline PR was full at ages 9–10. Longlining tends to be concentrated in deeper water not heavily fished by otter trawls.

The fishery-wide PR for the simulated two-gear fisheries varied considerably and in a manner consistent with the PR of the two fleets (Table 2). Full recruitment was at age 9 and was relatively flat-topped for fisheries with 100, 80, and 60% longline effort. For lower proportions of longline effort, full recruitment was at age 7 and the curves became progressively more dome-shaped. The variability in PR for ages 4–6 was higher for the higher proportions of longline effort. These results indicate the sensitivity of fishery-wide PR to the relative amount of effort by the respective gears.

Yield per Recruit

Estimates of $F_{0.1}$ varied from 0.26 for the 100% longline fishery down to 0.21 for the 40% longline fishery and then increased to 0.27 for a pure otter trawl fishery (Table 3). The sum of the standardized fishing effort was always greater than the $F_{0.1}$ for the mixed fisheries, since full recruitment to the two gears was not at the same age. The total effort varied little from 0.26 to 0.27. The highest yield per recuit was for a pure (100%) longline fishery (0.638 kg) and it declined steadily to 0.562 kg for a pure otter trawl fishery (0% longline effort). This decline in total catch at $F_{0.1}$ indicates that there is not a tonne-for tonne trade-off in catch allocations between the two gear sectors.

Given the virtually constant total effort and the decreasing trend in Y/R with decreasing longline participation, the fishery-wide CPUE also decreased in the same direction. However, the population fishable biomass (Y/F) varied inversely with the trend in $F_{0.1}$. This illustrates the difference between C/E and fishable biomass in a multigear fishery where the gears do not have full recruitment at the same age. The ratio between the two is max[Q_i]. At F_{max}, fishing mortality was higher for the pure longline fishery than for the pure trawl fishery. However, similar trends were found for total effort, Y/R, fishable biomass, and CPUE.

Under conditions of constant recruitment, mean population biomass per recruit was 4.167 kg in the pure otter trawl fishery and 5.567 kg in a pure longline fishery, an increase of 34% (Table 4). Fishable biomass per recruit for longlines increased from 1.584 to 2.478 kg, or by 56%. Fishable biomass per recruit for otter trawls increased by 50%. Fishable biomass for otter trawls was higher than that for longlines in all cases.

Interference

The interference of one gear on the other is illustrated by response surfaces of gear-specific yield as a function of F by

TABLE 3. Summary of yield per recruit calculations for simulated mixed fisheries with different levels of fleet participation. Fishable biomass per recruit (kg) is calculated as yield per recruit (kg) divided by F. CPUE is calculated as yield per recruit divided by effort. The percent otter trawl effort is 100% minus the percent longline effort.

		Effort					
% longline	F	Longline	Otter trawl	Total effort	Yield per recruit	Fishable biomass	CPUE
$F_{0.1}$							
100	0.258	0.258	0.000	0.258	0.638	2.47	2.47
80	0.237	0.206	0.052	0.258	0.620	2.62	2.40
60	0.220	0.156	0.104	0.260	0.603	2.74	2.32
40	0.209	0.105	0.158	0.263	0.588	2.81	2.24
20	0.240	0.053	0.214	0.267	0.574	2.39	2.15
0	0.273	0.000	0.273	0.273	0.562	2.06	2.06
F_{max}							
100	0.558	0.558	0.000	0.558	0.695	1.25	1.25
80	0.473	0.411	0.103	0.514	0.670	1.42	1.30
60	0.420	0.298	0.199	0.497	0.650	1.55	1.31
40	0.391	0.197	0.295	0.492	0.632	1.62	1.28
20	0.444	0.099	0.396	0.495	0.617	1.39	1.25
0	0.504	0.000	0.504	0.504	0.603	1.20	1.20

TABLE 4. Summary of long-term yield per recruit and biomass per recruit (kg) at $F_{0.1}$ in simulated mixed fisheries with different levels of fleet participation. Total yield and population biomass are highest for a pure longline fishery. Otter trawl fishable biomass always exceeded that for longlines.

	% longline effort					
	100%	80%	60%	40%	20%	0%
Total yield	0.638	0.620	0.603	0.588	0.574	0.562
Longline yield	0.638	0.471	0.327	0.201	0.093	0.0
Otter trawl yield	0.0	0.149	0.277	0.386	0.481	0.562
Population biomass	5.567	5.261	4.970	4.693	4.424	4.167
Longline fishable biomass	2.478	2.285	2.096	1.922	1.749	1.584
Otter trawl fishable biomass	3.090	2.875	2.662	2.455	2.254	2.064

each gear (Fig. 2). These yields represent steady-state conditions with constant recruitment. The axes are oriented to emphasize the response of yield to one gear to changes in F by the other. The surface of longline yield is much steeper along the otter trawl effort axis than is the otter trawl yield surface along the longline effort axis. The total yield surface is quite flat at Fs higher than 0.4 for both gears. The highest yields occurred with no otter trawl effort.

This point is further shown by the transition of yields to each gear in response to the addition of effort by one gear to a pure fishery by the other. Starting conditions were stable age distributions resulting from fishing at $F_{0.1}$ by one gear. The additional fishing effort corresponded to a fully recruited fishing mortality of 0.2 by the second gear. Subsequent recruitment was held constant. Adding otter trawl effort to the longline fishery resulted in a 59% decline in longline yield (Fig. 3). Adding longline effort to an otter trawl fishery resulted in a 23% decline in otter trawl yield.

The age and thus size of fish in the commercial catch is also affected by the mixture of fishing effort (Fig. 4). The modal age was eight in the exclusive longline fishery and five for the otter trawl fishery. The age composition of the catch in the mixed fishery was very similar to that of the otter trawl fishery.

Recruitment Variability and Catch Allocation

Recruitment and effort by gear were held constant in the examples presented above, and long-term equilibrium conditions were examined. Yield to the gear sectors would be constant through time under these conditions. However, recruitment is highly variable in the real world. The question addressed in this section is what happens to catch quotas and fleet effort if recruitment varies.

Year-class estimates for this stock varied from 108 million in 1968 to a low of 40 million in 1975 (Fig. 5). A trend is apparent with a period of higher than average recruitment in the mid-1960s, followed by a decline to the mid-1970s and another period of high recruitment. The latest three estimates are below the long-term average. The gear-specific fishable biomass trends are consistent with the recruitment trends, with a 2- to 3-yr lag (Fig. 6). The peaks in otter trawl fishable biomass preceded those of the longlines by about 3 yr, consistent with the difference in the age of full recruitment to the gears. This trend in fishable biomass was very similar regardless of the allocation regime used.

Depending on the allocation regime, there were differences in either fleet F or yield shares in response to changes in recruit-

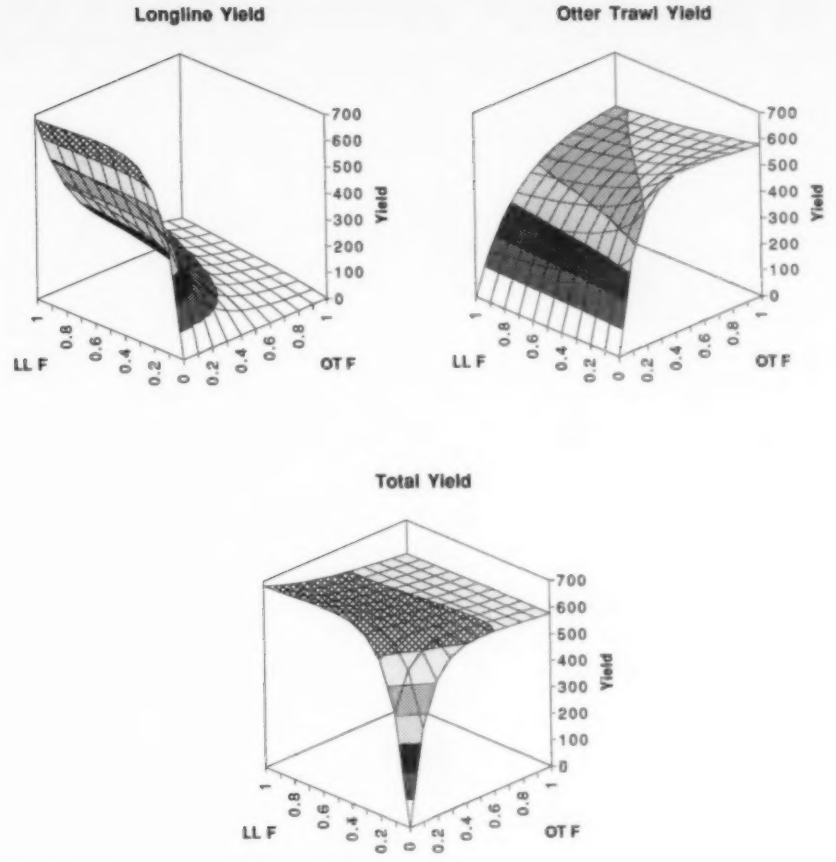

FIG. 2. Yield (g) per recruit for longlines, otter trawls, and in total as a function of fishing mortality (F) of the two fleets while fishing the same resource population. The longline yield surface is much steeper along the otter trawl F axis than the otter trawl yield surface is along the longline F axis. This indicates the higher sensitivity of longline yield to otter trawl F. The total yield surface is quite flat despite considerable variation in yield to the two fleets.

ment. Under the fixed percentage yield regime the fleet F varied inversely with the ratio of gear-specific fishable biomass to population fishable biomass. A decrease in the latter would result in a decrease in TAC. This would be shared equally between the gears. But the otter trawl fishable biomass would decrease before that of the longlines, since the trawlers catch fish at a younger age. Consequently, otter trawl effort would have to increase, while that for longlines would have to decrease. Thus, fishermen could be asked to fish harder when catch rates were in decline and fish less when catch rates were increasing. For example, the otter trawl catch rates would have declined between 1970–78, but for the same period, otter trawl fishing effort would have increased in order to catch the quota (Fig. 7). Through the subsequent period of increasing catch rates, otter trawl fishing effort would have declined.

On the other hand, under the fixed effort regime, yield by gear varied in direct proportion with fishable biomass (Fig. 8) and the relative proportions of the total catch varied through time. Fishermen would have to maintain constant effort regardless of changes in catch rate. Situations could arise where catch quotas for one gear could increase, while those for the other would decrease as a recruitment pulse passes through the population.

Discussion

Considering differences in partial recruitment of fish populations to competing fishing fleets is important when setting catch quotas. The relative effort exerted by the fleets will affect the population-wide PR and therefore target fishing mortalities. Long-term yields and fishable biomass will depend on the mix of gear types. The fleet that concentrates on younger fish can intercept incoming recruitment before it gets to the fleet that concentrates on older fish. Since the fleets exploit different age-groups, changes in fishable biomass due to recruitment variation are lagged, and the timing of fishing success will vary among fleets. I present and illustrate a methodology for investigating these interactions, for estimating the effects on long- and short-term yields and catch rates, and for predicting future trends given estimates of recruitment. The results are general with respect to the differences in partial recruitment among competing fleets. However, the results are not necessarily

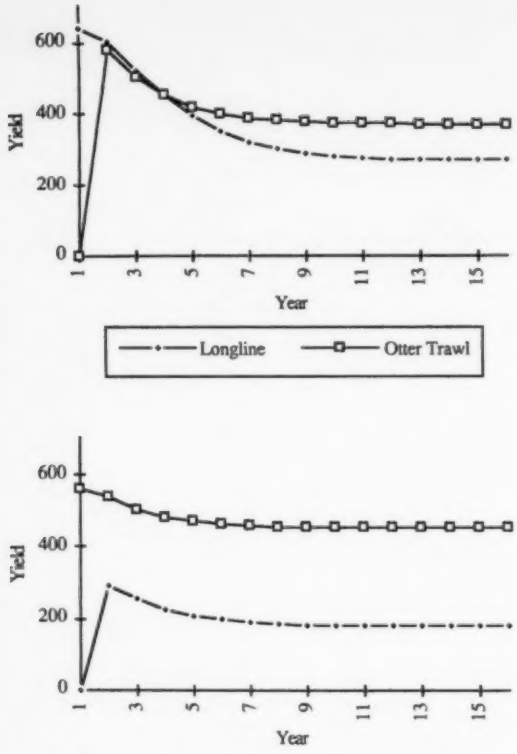

FIG. 3. Catch by gear following the addition of effort by one gear ($F = 0.2$) to a pure fishery at $F_{0.1}$ by another. In the upper panel, otter trawl effort was added to a longline fishery. The reverse was the case in the lower panel. Longline yield is considerably more sensitive to the addition of otter trawl effort than otter trawl yield is to the addition of longline effort.

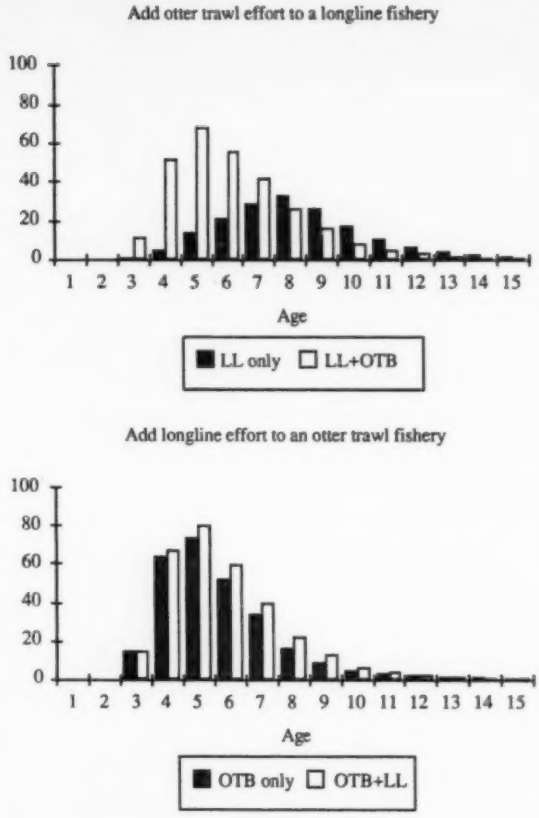

FIG. 4. Catch-at-age before and 15 yr after (open histograms) the addition of effort by one gear ($F = 0.2$) to a pure fishery by the other. In the upper panel, otter trawl effort was added to a longline fishery. The reverse was the case in the lower panel. Adding otter trawl effort to a longline fishery caused a large shift in age composition of the catch toward younger fish. Adding longline effort to an otter trawl fishery resulted in a much smaller shift in age composition toward older fish.

general with respect to the fishing gear used by the fleets. The fleet-specific results may vary if the fleets change either their fishing locations or the configuration of their gears so as to change the partial recruitment.

The two fleets examined in this case study had very different PR. The otter trawl fleet exploits relatively younger fish, with the ages 6–7 being fully recruited, and PR declined for older fish. This decline in PR with age is probably a consequence of the lower availability of older fish on the grounds occupied by these vessels. Their effort is concentrated on shallow banks, while the older fish tend to occupy deeper waters off the banks. Otter trawl PR could be shifted toward older fish by displacement of effort to deeper waters and by increasing mesh size. Alternatively, the longline fleet exploits older fish with full recruitment to the gear at ages 9+. While quantitative data on size selection of hooks and on hook size usage by this fleet are lacking, it is probable that, during the period examined, the longline gear selected older fish than the otter trawls (R.G. Halliday, Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, personal communication). Longline effort is also directed toward deeper waters where older fish are more abundant. It should be noted that these two gears have difficulty fishing the same grounds simultaneously, since longlines are anchored on the bottom and thus are susceptible to destruction by passing trawls. It is entirely possible that in the absence of trawlers, the longlines would be placed on the shallow banks and the longline fleet could switch to smaller hooks, thus shifting PR toward younger fish.

The population-wide PR depends on the mixture of fishing effort by the fishing fleets. Variations in annual fishing effort by fleet will result in changes in the relative exploitation rates of different age-groups. This will have an effect on yield per recruit and fishing mortality targets of management as well as on the accuracy of PR used to make TAC projections. It may also explain at least part of the interannual variation observed in fishery-wide PR noted by Rivard and Foy (1987).

Fisheries managers may find useful information in these calculations for deciding on target combinations of fishing effort among fleets. In the case study presented, long-term yield, CPUE, and total population biomass would be highest for a pure longline fishery. Fishable biomass was highest for the 40% longline fishery. Total standardized fishing effort would be highest for a pure otter trawl fishery. The trend in nominal fishing effort would depend on the fleet catchabilities. Otter trawl fishable biomass was always higher than that for long-

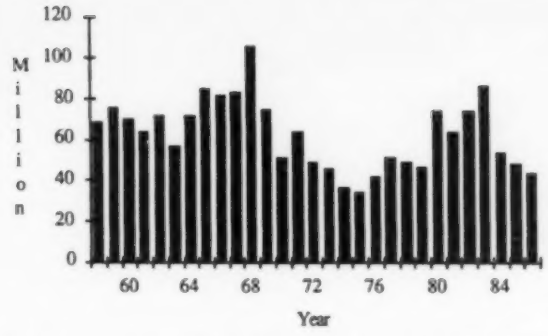

FIG. 5. Recruitment estimates of age 3 cod from the 4VsW management unit. Recruitment was high in the mid-1960s and the early-1980s and low in the mid-1970s and mid-1980s.

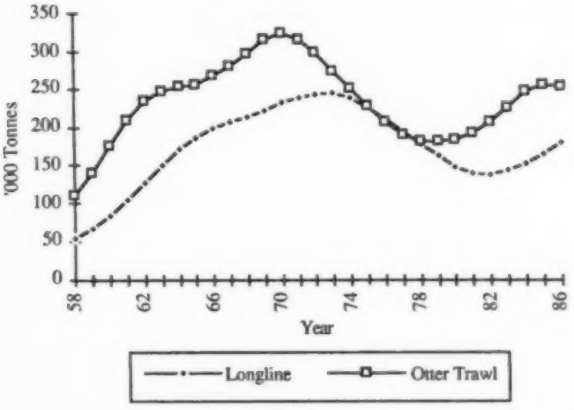

FIG. 6. Trends in fishable biomass for longliners and otter trawlers fishing cod in the 4VsW management unit under a fixed percent yield allocation regime. Trends in longline fishable biomass lag behind those of otter trawls by approximately 3 yr which corresponds to the difference in age at full recruitment.

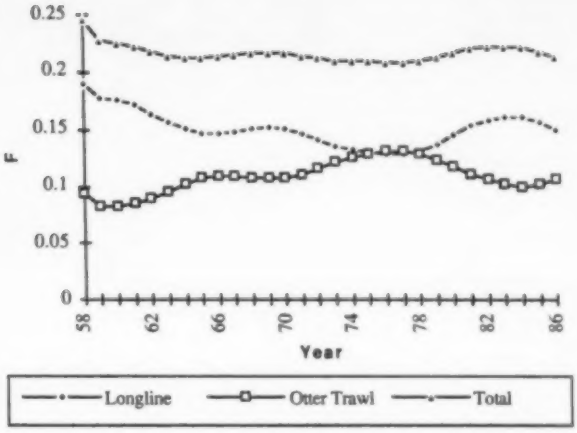

FIG. 7. Trends in fishing mortality by fleet sector while fishing cod in the 4VsW management unit under a fixed percent yield allocation regime. Gear-specific fishing mortality varied inversely with the ratio of fleet fishable biomass to population fishable biomass.

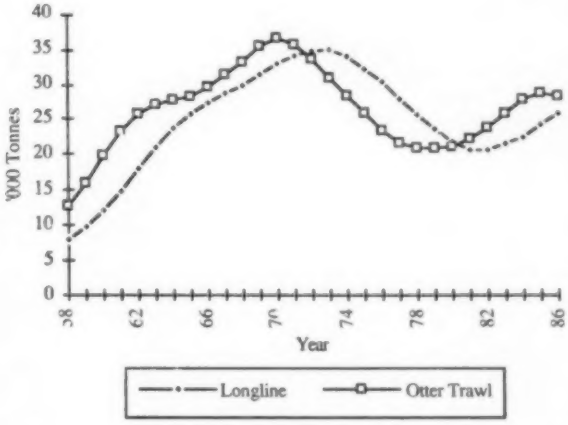

FIG. 8. Trends in yield by fleet sector while fishing cod in the 4VsW management unit under a fixed fishing mortality allocation regime. Fleet yield varied directly with fleet fishable biomass because fishing mortality for each gear was constant.

lines, since younger fish are exploited by this gear. This would contribute to a comparative advantage for vessels with otter trawls when competing with vessels with longlines for a limited catch. With the addition of data on average crew sizes and trip lengths, one could estimate the employment consequences of different effort combinations. Cost and price data would allow the estimation of the economic consequences (O'Boyle et al. 1991).

The results of this study indicate that the age structure of catches by fleets should be considered when quota transfers are made. The current groundfish management regime in eastern Canada allows for catch quota transfers among fleets in mid-season if one fleet is unlikely to catch its quota. Catch may fall short of the quota in the short term due to several factors including reductions in fleet effort, overestimates of the TAC, and changes in fish availability. However, the long-term yield at $F_{0.1}$ in a pure longline fishery was estimated to be higher than that for a pure otter trawl fishery. Thus, a tonne of longline catch is not equivalent to a tonne of otter trawl catch. While the transfer of quota from the longline fleet to the otter trawl may satisfy a short-term objective, for example, to catch the annual TAC, it may lead to reduced abundance for the longline fleet in future years and therefore make catching subsequent quotas more difficult. Furthermore, in order to maintain catches at $F_{0.1}$, a quota transfer also infers an effort transfer. This may necessitate a reevaluation of the resource sharing arrangement and a reevaluation of the target fishing mortality, since the fishery-wide PR will change.

The addition of otter trawl effort to a longline fishery clearly has different implications than the reverse situation. In the former case, when only longlines were used, the younger ages (4–6) would not be heavily fished and would be at high abundance. The vessels with otter trawls would experience high catch rates and apparent good fishing conditions upon their arrival. Continued fishing with otter trawls would reduce the abundance of these age-groups before they recruit to the longline fishery, leading to a decline in longline yield for the same

effort. Quota shortfalls could result. In the alternative case the new entrants (longliners) would exploit age-groups that had survived otter trawl fishing. Consequently, the catch by otter trawls would decline by a much smaller amount and quota shortfalls would be less likely.

Year-class variation will affect fleet fishing effort in the quota allocation system used in eastern Canada. The management strategy is to fish the stocks at a constant fishing mortality of $F_{0.1}$. The yield sharing arrangement is to allocate the TAC among the fleets based on a fixed proportion. However, as year-class strengths vary, the relative biomass available to the fleets will also change. Thus, fleet fishing mortality and effort will also vary. Fishermen may have to fish harder when catch rates are in decline in order to catch their allocations. There may be situations when the existing fishing fleets may not be capable of catching their quota simply because there is not enough capacity. Alternatively, they may have to fish less when catch rates are increasing. The methodology presented here may be used to predict fishable biomass by fleet for the period of TAC projections. The information would be useful for managers in anticipating the rate at which fleet quotas could be caught.

This methodology could also be used to estimate fleet quotas if the allocation strategy was to keep fleet effort constant. In this case, the fleet quotas would follow the fleet fishable biomass.

The difference between the fully recruited fishing mortality (F) and the sum of standardized efforts in mixed-gear fisheries has important implications regarding the use of aggregated catch rates as indices of stock abundance. CPUE is commonly used as an index of stock size (equation 1). However, only when the gears have full recruitment at the same age will total catch divided by the sum of fishing effort across gears be an unbiased estimate of fishable stock size. A solution to this problem is to use separate catch rate series from the different fleets as independent indices of stock status.

Acknowledgements

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Ontogenetic Changes in Prey Selection and Visual Acuity of the Yellow Perch, *Perca flavescens*

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Age-0 yellow perch, *Perca flavescens*, shift from pelagic to demersal waters of Oneida Lake, New York, between late June to mid-July, when they reach standard lengths of 24–31 mm. The timing of this habitat shift coincides with the size range over which yellow perch achieve a degree of visual resolution that nearly equals that of adult yellow perch, from 174 min of arc in newly hatched larvae to 9–12 min in adults. This visual improvement is reflected in the yellow perch's diet, which consists of an increasingly wider range of prey sizes and types. If twin cones are counted as functionally separate photoreceptors, there is a significant improvement of the calculated visual acuity in larval fish with lenses <1 mm in diameter but not in older fish with larger lenses. During its rapid growth phase the most optimistic calculation of visual acuity in a young yellow perch is insufficient to explain the feeding success necessary at this time. We suggest therefore that young yellow perch spend more time in search of prey than their adult counterparts.

Les perchaudes, *Perca flavescens*, d'âge 0+ du lac Oneida (New York) migrent de la masse d'eau vers les fonds entre la fin juin et la mi-juillet, au moment où leur longueur standard se situe entre 24 et 31 mm. Ce changement d'habitat coïncide avec la gamme de taille à laquelle les jeunes perchaudes parviennent à une acuité visuelle presqu'équivalente à celle des adultes; la résolution qui est de 174 min d'arc chez les larves nouvellement écloses est de 9 à 12 min chez les adultes. Cette amélioration de la vision se répercute sur le régime alimentaire des perchaudes dont la gamme de tailles et de types de proies s'élargit. Si les deux cônes de vision sont considérés comme étant des photorécepteurs fonctionnels distincts, on note alors une amélioration appréciable de l'acuité visuelle calculée des poissons larvaires dont le diamètre du cristallin est inférieur à 1 mm, mais non chez les poissons plus âgés à cristallins plus grands. Pendant la phase de croissance rapide, même les calculs les plus favorables de l'acuité visuelle des jeunes perchaudes ne suffisent pas à expliquer le degré de succès de la recherche de nourriture qui est alors nécessaire. Nous supposons donc que les jeunes perchaudes consacrent plus de temps que les adultes à la recherche de nourriture.

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A fish's feeding success and diet selectivity are affected by a variety of factors, including prey size (Wong and Ward 1972; Furnass 1979; Hansen and Wahl 1981; Hunter 1981; Bence and Murdoch 1986; Confer et al. 1990), prey vulnerability (Vinyard 1980), prey motion (Zaret 1980), prey visibility (Zaret and Kerfoot 1975; O'Brien 1979; Hairston et al. 1982; Breck and Gitter 1983), gape limitations of the planktivore (Wong and Ward 1972), and prey diversity and abundance (Confer et al. 1990). Low visual acuity is thought to impose constraints on the ability of small fish to choose prey (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985). Larval fish are often size-selective feeders and generally select prey smaller than might be predicted from models of apparent prey size (Furnass 1979; Hansen and Wahl 1981; Bence and Murdoch 1986; Confer et al. 1990).

The visual system of most fishes is functional just before, or soon after, the yolk sac is absorbed (Blaxter 1988). In some species, such as the yellow perch, *Perca flavescens*, hatchlings have a differentiated retina containing functional cone cells (killifish (mummichog), *Fundulus heteroclitus*, Hollyfield 1972; European perch, *Perca fluvialilis*, Ahlbert 1973; threespine stickleback, *Gasterosteus aculeatus*, Ekstrom et al. 1983; guppy, *Poecilia reticulata*, Kunz and Ennis 1983; zebrafish, *Brachydanio rerio*, Branchek 1984; Branchek and Bremiller 1984). The retina of yellow perch hatchlings contains a class of ultraviolet-sensitive cones not found in the adult (Loew and Wahl 1991) and very few rod cells that are identifiable only by transmission electron microscopy (Ahlbert 1976). Larval cone

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cells are smaller and morphologically distinct from those of juvenile or adult fish (Wahl 1990; Loew and Wahl 1991).

The morphological differences between larval and adult fish eyes suggest that functional refinements appear sequentially during development (Wahl 1990; W.N. McFarland and C.M. Wahl, unpublished data on the French grunt, *Haemulon flavolineatum*). Changes in patterns of prey selection as young fish grow are probably associated, at least in part, with these developing refinements of the eye. This study reports ontogenetic changes in the photopic acuity of yellow perch and relates changes in behavior and prey selection to development of visual acuity. To this end, we examined changes in the diet, visual acuity, anatomical development of the eye, and feeding behavior of yellow perch from hatchling to adult.

Materials and Methods

Eggs of the yellow perch were collected from Oneida Lake, New York, and hatched in the laboratory in May of 1988–90. Hatchlings were transferred to 80-L aquaria. Yellow perch were fed a daily meal of Oneida Lake zooplankton strained through a 1.0-mm-mesh sieve (zooplankton length <1.5 mm). Aquarium-reared fish were examined from the time of hatching until they reached 20 mm standard length (SL). Yellow perch of a range of sizes from 20 up to 240 mm SL were either seined nearshore or trawled from the bottom waters of Oneida Lake.

Diet of Posthatching Yellow Perch

To determine the diet of yellow perch <20 mm SL, fish were collected at daily intervals between 10:00 and 15:00 0.1–2 km offshore using either a 0.5- or a 1-m net equipped with either 150- or 750-μm nylon mesh netting. Fish were captured from the surface to a depth of 3 m from May 9 to June 12, 1989. Individuals were narcotized with tricaine methanesulfonate (MS 222) before preservation in 10% formalin. Standard lengths were measured to the nearest tenth of a millimetre. Stomach contents of individual fish were removed and placed in a grid-etched Sedgewick–Rafter cell. Because the stomach and intestines of yellow perch <20 mm SL are indistinct at the gross dissection level, all prey in the entire digestive tract were counted and measured with electronic calipers interfaced with a microcomputer (Mills and Confer 1986). Each prey item was measured from the anterior margin of the head to the base of either the tail spine of *Daphnia* or the caudal setae of copepods. Fragments of individual prey were usually recognizable for measurement. We assumed that fish >20 mm had stomach contents similar to those found by Hansen and Wahl (1981).

Habitat Shift from Pelagic to Demersal

Age-0 yellow perch were collected weekly from mid-May to early August of 1979, 1980, and 1990 to determine when these fish shifted from the pelagia to the demersal habitat. Collections were made at 1 and 3 m using Miller samplers, from 5 to 7 m using a semiballoon trawl, and over the lake bottom with an otter trawl. All collections were made during daytime (10:00–15:00).

Examination of Whole Eyes and Retinas

Eye and lens diameters were measured in fresh specimens. Retinas of individual fish were dissected away from the pigment epithelium (PE) and floated, photoreceptor side up, onto clean glass slides using the eye's own vitreous. Two slips of paper were used to support the cover slip over the retinal specimen, thus ensuring that retinas were not distorted by pressure. The retinal cone mosaic was photographed and cone density was determined using an Olympus BH-2 microscope equipped with Nomarski differential interference contrast optics. Cone cell densities were counted in only the central posterior part of the retina, as this corresponds to the forward visual field, where fish direct their strikes (Hairston et al. 1982; Browman et al. 1990) and because cone cell densities vary across other regions of the retina (Collin and Pettigrew 1988; Browman et al. 1990; Wahl 1990).

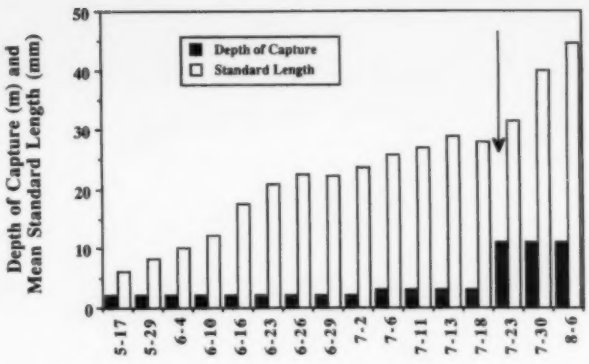

FIG. 1. Depth of capture and mean standard length of age-0 yellow perch in Oneida Lake for the year 1979. The arrow indicates a demersal shift. Similar results were found for at least two other years.

Calculations of Visual Acuity

When cones were clearly in focus and obscuring pigments were absent, we counted the number of cones per 0.1 mm^2 in 6–12 patches per retina. The maximum possible photopic visual acuity of a fish is called the minimum separable angle (MSA), defined as the smallest angle between two points in space that the eye can distinguish as separate. It was calculated using the formula derived by Tamura (1957):

$$\sin(\text{alpha}) = 1/f \cdot 0.2/\sqrt{n}$$

where alpha = MSA (radians), f = focal length (millimetres), and n = cones/(0.1 mm)^2. Tamura assumed a shrinkage value of 0.25 in his formula, but this factor was dropped from our calculations because we used fresh, not fixed, retinas.

Scanning electron microscopy was used to determine the presence or absence of rods and to examine more closely the morphology of the inner and outer segments of the cones. Retinas were immersed in a solution of 4% paraformaldehyde and 1% glutaraldehyde and buffered with Sorensen's phosphate to a pH of 7.0 and 350 mosmol. After 24 h, the retinas were rinsed with 0.15 M Sorensen's phosphate buffer, to which 1 mM calcium chloride was added, and postfixed for up to 2 h in 1% osmium tetrachloride and potassium dichromate. Retinas were then dehydrated in a series of ethanols and critical-point-dried in a Samdri automatic CPD machine. Dried retinas were fractured by hand, mounted on stubs, and coated with gold–palladium. They were examined using an AMR 1000 scanning electron microscope.

TABLE 1. Diet of young yellow perch in Oneida Lake, New York, May–June 1989.

Date	Number of fish examined	Mean fish standard length (mm)	Mean number of organisms/fish				
			Nauplii	Cyclopoids	Calanoids	Daphnids	All prey
May 17	17	6.7	2.2	3.0	0.9	0.0	6.1
May 18	6	6.8	0.3	3.5	0.2	0.0	4.0
May 19	5	7.3	0.0	3.0	0.0	0.0	3.0
May 22	9	8.3	0.0	3.7	0.9	0.0	4.6
May 24	12	7.9	4.8	3.3	1.3	0.0	9.4
May 26	17	9.6	0.2	4.1	0.2	0.0	4.5
May 28	12	9.2	2.1	13.5	1.8	0.2	17.5
May 29	29	9.1	0.7	6.5	1.1	0.3	8.7
May 31	72	11.2	1.8	3.6	3.9	0.0	9.4
June 1	30	11.4	0.3	2.2	5.5	0.1	8.1
June 2	49	11.4	0.2	2.6	4.0	0.0	6.8
June 4	28	12.2	0.1	5.9	9.2	0.3	15.5
June 6	63	12.5	1.6	17.1	5.4	0.1	24.2
June 7	57	13.2	0.6	3.7	6.6	0.1	11.1
June 8	25	14.1	0.5	2.2	9.1	0.1	11.8
June 11	35	15.6	0.1	1.4	22.9	1.1	25.5
June 12	54	15.5	0.1	17.7	11.4	0.9	30.1

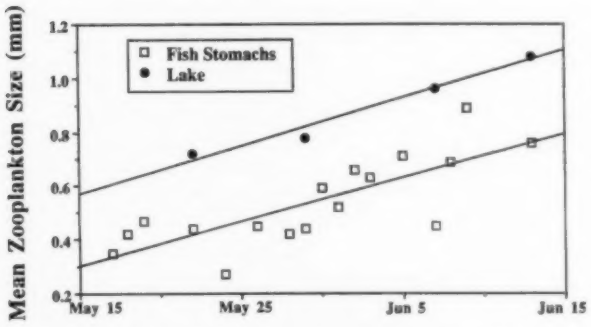

FIG. 2. Average size of zooplankton eaten by very young yellow perch from mid-May to mid-June during 1989 and the average size of zooplankton available in Oneida Lake at this time.

Results

Ontogenetic Habitat Shift

Age-0 yellow perch hatch in mid-May and are predominantly found in trawls taken at depths of less than 2.5–3 m until midsummer, when they become demersal (Fig. 1). In 1979, young yellow perch shifted from the pelagia to the demersal habitat over a 5-d period when they reached 28–31 mm SL and 62–67 d of age. In other years, young yellow perch shifted to the demersal habitat earlier but over a similar range of fish sizes. In 1980, these fish switched their habitat preference to deep water when they were 24–27 mm SL. This pattern was consistent with observations in 1990, when the shift occurred between 26 and 30 mm SL, corresponding to ages of 46–50 d. Age differences at similar standard lengths may be due to a number of factors, including variations in prey recruitment and temperature from year to year.

Prey Selection

Stomach contents of 520 yellow perch <15.5 mm SL collected during May and June of 1989 were examined (Table 1). Over this period, yellow perch increased in average size from 6.7 to 15.5 mm SL. The first food of newly hatched yellow perch was generally dominated by the cyclopoid *Cyclops biscupidatus*, although nauplii and calanoid copepodites were also eaten. The predominant calanoid copepods were *Diaptomus* spp. and *Epischura lacustris*. Although daphnids (primarily *Daphnia pulex*) were abundant in Oneida Lake during the study period, they did not appear in the stomachs of yellow perch until the fish were about 9 mm SL. As seen in Fig. 2, they are consistently eating prey smaller than the average size available in the lake, but they progress to larger average prey sizes as they grow, and as the average size of lake zooplankton begins to decline.

There was an increase in the overall size range of prey selected as fish grew, up to 15.5 mm SL, as indicated in Fig. 3. For fish >15.5 mm SL, the 1979 data of Hansen and Wahl (1981) were reexamined and incorporated into Fig. 3. In their study, stomach contents of 160 individuals ranging from 21 to 52.3 mm SL were examined. The maximum body size of zooplankton eaten increased with fish size, although the smallest sizes consumed tended to remain constant. This indicates an increasing range in choice of prey by older, larger fish. There was considerable variability among individual yellow perch in the sizes of prey chosen, but they generally preferred prey smaller than they were actually capable of ingesting.

Lens Diameter, Cone Density, Fish Size, and Visual Acuity

For yellow perch ranging 5.9–80 mm SL, lens diameter increases with fish size (Fig. 4). Cone density, as in other fishes (Fernald 1988), is inversely related to fish size, with the smallest yellow perch having densities four times those observed in adults (~39 versus 9.5 cones/0.01 mm^2). The MSA of newly hatched larvae is 174 min, or nearly 3°, if it is assumed that each cone cell functions independently as a photodetector. The MSA declines exponentially and for yellow perch >200 mm SL is 9–12 min of arc.

Development of Cone Cells

Several morphological features of cones change as yellow perch grow (Fig. 5): (1) the size of the cone cell increases, (2)

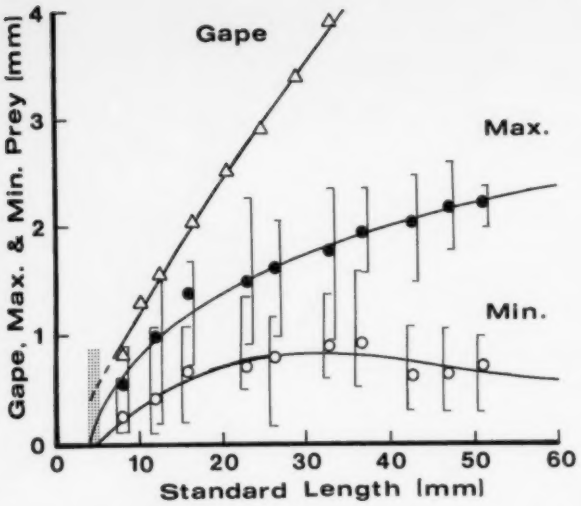

FIG. 3. Interrelationships between mouth size and the average maximum- and minimum-sized prey consumed by different sizes of young-of-the-year yellow perch. The gape versus standard length (open triangles) was computed from data in Wong and Ward (1972). The mean size of the largest (solid circles) and smallest prey eaten (open circles) include the range (vertical bars). A total of 685 fish were examined and categorized in 5-mm increments of standard length for data reduction. Data are plotted as the mean standard length within each 5-mm size category. The maximum-sized prey consumed was significantly different from the minimum-sized prey eaten over all size categories ($p \leq 0.01$). The stippled area represents the size range of post-hatchlings. The regression line for maximum prey eaten is $y = -0.18 - 0.005x^2 + 0.00004x^3$; $R^2 = 0.86$.

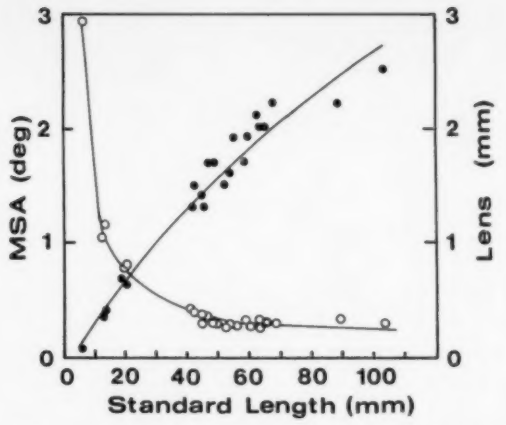

FIG. 4. Regression of visual acuity (MSA) and lens diameter against standard length of young-of-the-year yellow perch. Open circles indicate the minimum separable angle in degrees; solid circles indicate lens diameters.

the relative proportions of the inner and outer segments are modified, and (3) the cone nuclei change from columnar to round in shape.

The cone mosaic (as originally described by Eigenmann and Shafer 1901) also changes with increasing body size (Fig. 6). In hatchlings (Fig. 6a) the untwinned cones are hexagonally packed and lack the geometric order characteristic of mature perch. When twinning of cones commences, about 6 d after hatching, the square mosaic characteristic of adult fish begins to take shape (Fig. 6d; and Wahl 1990). It appears first centrally, and then spreads peripherally, where new cones are being added. In this peripheral zone of cell addition, there is a row mosaic. Young-of-the-year fish have additional small ultraviolet-sensitive cones at the corners of each square, forming "complete squares" that degenerate during midsummer and fall, leaving a "simple square" mosaic (Loew and Wahl 1991). The ultraviolet cones enhance foraging on zooplankton (Loew et al. 1993).

Discussion

Natural History

Postlarval yellow perch move from pelagic to demersal waters of Oneida Lake between late June and mid-July, when they are 24–31 mm SL. This movement occurs when the visual acuity of yellow perch, as indexed by the MSA, approaches adult values. Light levels during daytime are low in the demersal habitat (<2 lx) compared with intensities in the near-surface waters (Mills et al. 1984). Transition to the demersal habitat results in diminished searching ability due to decrements in light intensity (O'Brien 1979), for which improved visual acuity may partially compensate.

The accelerated improvement in resolving power during the first 2 mo of growth in yellow perch is also observed in rainbow trout, *Oncorhynchus mykiss* (formerly *Salmo gaidneri*) (Rahmann et al. 1989). In addition to an increase in the average size of prey taken (Furnass 1979; Hansen and Wahl 1981; Mathias and Li 1982; Mills et al. 1984), the prey capture success of yellow perch >27 mm SL is nearly 100% (Mills et al. 1984). Smaller yellow perch have lower capture rates, but whether this is attributable to less ability to detect and visually direct attacks at prey or merely from difficulties in handling prey is not clear.

Because yellow perch are visual feeders, one would suppose that foraging is necessarily facilitated by eye development, especially in the early life stages when retinal development is most rapid and visual resolution low. Initially, however, yellow perch are gape limited. From hatching at around 7–8 mm SL and up to a size of about 10 mm SL, the largest zooplankters they consume are slightly smaller than their maximum gape (Fig. 3).

As visual acuity rapidly improves (Fig. 4), the detection distance also increases (Fig. 7). It follows that the search volume in which younger yellow perch can detect a given size of plankton is comparatively small and likely limits their potential prey encounter rate relative to that of larger juveniles. Until they reach a size of about 30 mm SL (end of the transition phase), their prey is *detectable* at maximum distances <20 cm. The distance at which they actually *recognize* prey is certainly smaller and, in fact, the data in Fig. 7 likely exceed the actual field detection abilities because Oneida Lake is eutrophic, typified by diminished water clarity, which did not enter into our calculations. Other studies have shown that the reactive distance of sunfish is diminished with reduced water clarity and that reactive distance to the same size prey increases with fish size (O'Brien 1979). Nevertheless, young yellow perch are capable of rapid digestion of large numbers of prey. This is an essential feature because a rapid rate of growth favors survival (Mills and Forney 1988; Mills et al. 1989), and the smallest

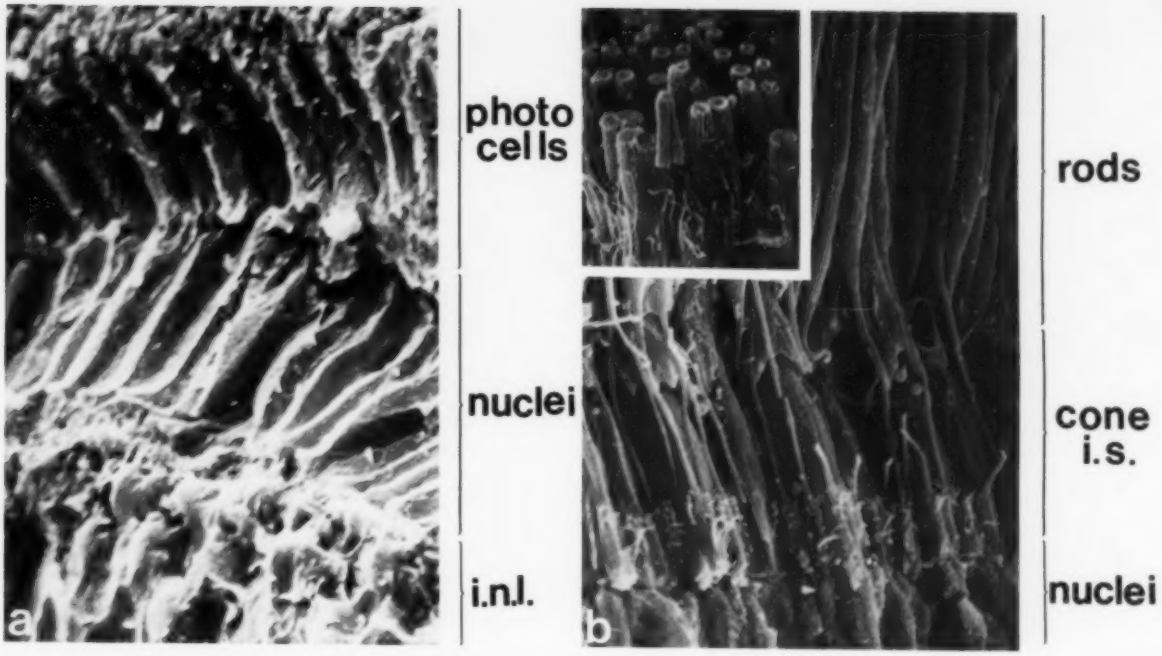

FIG. 5. Scanning electron micrograph of (a) larval and (b) adult yellow perch cone receptors. Note especially the elongated cone nucleus present in the larval retina. That of the adult is more rounded and less columnar in appearance.

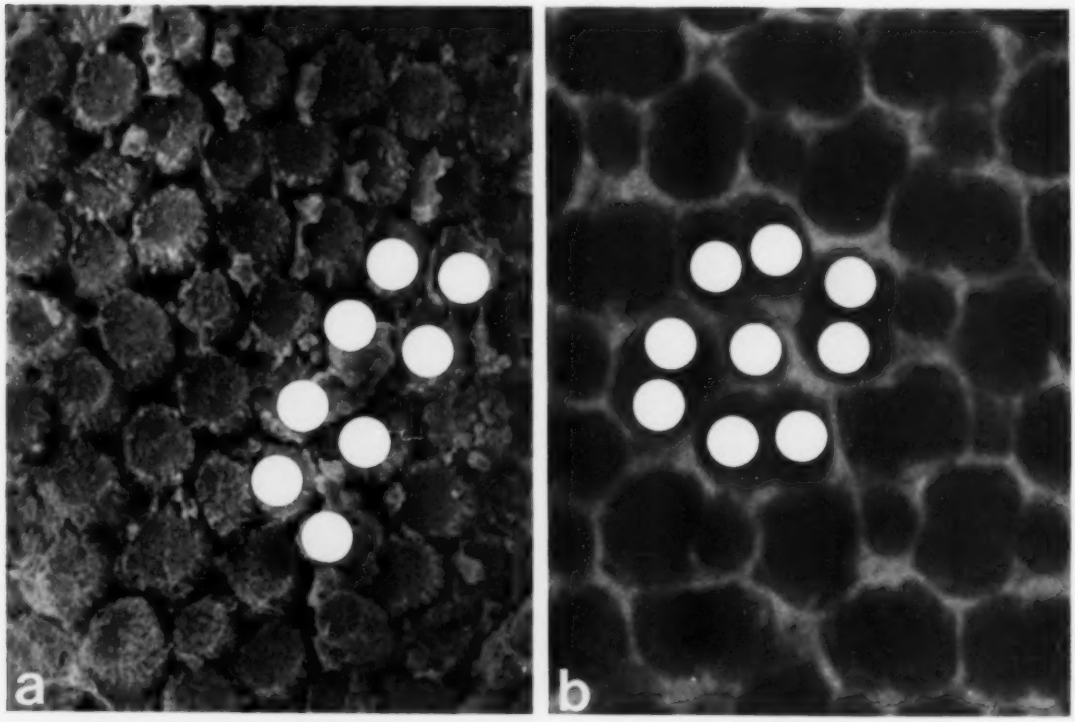

FIG. 6. Photoreceptor cone mosaics in (a) larval (scanning electron micrograph and (b) adult (light micrograph) yellow perch. The white dots indicate the center of cone photoreceptor outer segments and help to show the differences between the row and square mosaic configuration. For clarity, not all cones have been labelled.

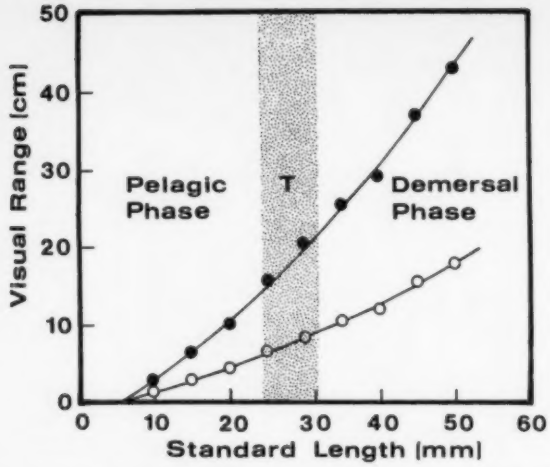

FIG. 7. Visual range versus standard length for young-of-the-year yellow perch. Solid circles indicate values calculated for the average maximum-sized prey eaten at increasing standard lengths; open circles indicate values for the average minimum-sized prey eaten. Prey sizes were obtained from the data in Fig. 3. The visual ranges are calculated for ideal conditions and do not take into account, for example, absorption and scattering by particles that might be present in the water column (see text). The stippling covers those standard lengths over which young-of-the-year yellow perch switched from pelagic to demersal habitats in Lake Oneida.

yellow perch are most vulnerable to predation (Forney 1980). Young yellow perch are able to optimize their digestive efficiency by selecting appropriate prey sizes (Mills et al. 1989), a circumstance that certainly must enhance growth rate, and yet this appears to be accomplished when the visual sense is least developed.

These observations suggest either that (1) vision is not critical for feeding success in young yellow perch or (2) young fish must spend considerably more time searching for prey than older fish. Observations of fish kept in total darkness reveal that young yellow perch will not or cannot eat in the absence of light (C.M. Wahl, personal observation; Loew et al. 1993). Thus, they may be spending proportionately more hours actively searching for prey than their adult counterparts.

Morphological Measurements

We examined the relationship between visual acuity, as measured morphologically, and fish size. There are two principal determinants of acuity within the eye: (1) the optical pathway and (2) cone density. Optimal cone density is not necessarily matched to the actual resolving power of the optical pathway. In fact, contrast sensitivity is enhanced by decreasing the retinal grain relative to optical resolution (Fernald 1988). In humans, the retinal resolving capacity closely matches the optical resolution (Rodieck 1973), producing a total resolving power of up to 60 lines/degree. However, in the fishes examined so far, the retina appears to limit the resolving power of the eye. The perch *P. fluviatilis* has a cornea–lens resolution of 3.1 lines/degree but a calculated retinal resolving power of only 2.7 lines/degree (Otten 1981).

As lens diameter in *P. flavescens* increases from 0.2 to 5.0 mm, visual acuity improves exponentially. The minimum separable angle of newly hatched larvae was 174 min of arc, or nearly 3°; for adults it was 9–12 min of arc. The impact of these figures on the visual range, or search volume, in juveniles versus adults is schematically depicted in Fig. 7. Otten's (1981) findings for *P. fluviatilis* are not in agreement with ours. He calculated the best retinal resolving power for adult perch to be only 22 min of arc. There is good agreement, however, between our results and those reported for *P. fluviatilis* by Guma (1978), who found similar cone densities. Differences in technique may account for the discrepancy with Otten.

We questioned whether individual members of newly twinned cone pairs in very young fish function as separate receptors or not. In the absence of electrophysiological data, we assumed that twin cones function as two separate cells if the calculated visual acuity consequently improves. A yellow perch gains no significant improvement in visual acuity, as indexed by the MSA, if the twin cones are counted as separate receptors when the lens diameter is ≥ 1 mm. There is, however, substantial improvement in calculated acuity if the twin cones are treated as separate units below a lens diameter of 1 mm. It is possible that twin cones function as separate units in yellow perch <25 mm SL, maximizing their ability to forage on plankton. In larger yellow perch, with larger lenses but less densely packed cones, twin cones most likely function as single units, as visual acuity depends principally on the optical quality of the eye, not receptor density. Anatomical observations lend support to the possibility that twin cones function differently during early versus later phases of eye development. The membranes separating members of larval twin cone pairs are smooth and straight, with few synaptic features or undulations, while adult cones have all these features (Ahlbert 1973; C.M. Wahl, personal observations). That twin cones may function independently during early development of yellow perch poses important questions in visual physiology and in fish ecology that need to be considered. Virtually all marine fishes and numerous freshwater species are obligate diurnal planktivores as posthatchlings (Leis 1991). Given the precariousness of these early life stages with respect to starvation, growth, and predation (Hunter 1981) and their low visual resolving abilities, the possible improvement in vision achieved by a separation of twin cone function, however fleeting, may spell the difference between survival or an early death.

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Aggregating Behavior of Ovigerous Female Red King Crab, *Paralithodes camtschaticus*, in Auke Bay, Alaska

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Ovigerous female red king crab, *Paralithodes camtschaticus*, monitored with ultrasonic biotelemetry, displayed distinct seasonal shifts in habitat use and bathymetric distribution. Crab occupied deep water between June and mid-November, relatively shallow water between mid-November and early March, and returned to deeper water prior to molting and mating in spring. Females were nonrandomly distributed within both depth ranges but seasonally changed their behavior and degree of association with conspecifics. Tagged crab were more aggregated (lower mean nearest neighbor distances) in winter than in summer and fall (*F*-test, $P < 0.01$). Winter SCUBA observations supported these results; females of mixed age-classes formed dense aggregations in shallow water, and crab exhibited "podding" behavior similar to that reported for younger age-classes. Adult males were associated with aggregations during late winter and early spring. Observations from the manned submersible *Delta* indicated that females were nonrandomly distributed in the deepwater habitat as well and probably formed loosely knit feeding aggregations there. Seasonal changes in aggregation densities and benthic areas used were the same during the two consecutive years of this study.

Des femelles ovigères de crabe royal, *Paralithodes camtschaticus*, contrôlées par biotélémétrie ultrasonique ont présenté des modifications saisonnières distinctes touchant l'utilisation de l'habitat et la répartition bathymétrique. Les crabes se trouvaient en eau profonde entre juin et la mi-novembre, en eau relativement peu profonde entre la mi-novembre et le début de mars et en eau profonde au printemps, avant le début de la mue et de l'accouplement. Les femelles étaient réparties de façon aléatoire dans les deux gammes de profondeur, mais leur comportement et leur degré d'association avec les crabes de même espèce se modifiaient de façon saisonnière. Les crabes marqués étaient plus concentrés (plus faible distance moyenne entre individus) en hiver qu'en été et en automne (test *F*, $P < 0{,}01$). Des observations faites l'hiver en plongée autonome confirment ces résultats : les femelles des diverses classes d'âge formaient des concentrations denses en eau peu profonde et les crabes formaient des "groupes" comme cela a été signalé pour les individus plus jeunes. Les mâles adultes étaient associés aux concentrations à la fin de l'hiver et au début du printemps. Des observations faites à partir du submersible habité *Delta* ont permis de montrer que les femelles n'étaient pas dispersées de façon aléatoire dans les eaux plus profondes et qu'elles y formaient probablement des concentrations d'alimentation à liens étroits. Les changements saisonniers de la densité des concentrations et des zones benthiques utilisées se sont avérés être les mêmes au cours des deux années consécutives de l'étude.

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Red king crab, *Paralithodes camtschaticus* (Tilesius), undergo ontogenetic shifts in depth distribution, habitat use (Wallace et al. 1949; Stone et al. 1992), and the dispersion of individuals within age-classes (Powell 1974; Dew 1990). Following metamorphosis from the larval form, juveniles adopt a solitary and cryptic existence during the first year of benthic life, occupying protected refuges in the intertidal and shallow subtidal regions (Sundberg and Clausen 1977). In contrast, subsequent age-classes (1–4 yr old) often form dense aggregations in shallow sublittoral zones (Bright et al. 1960; Durham 1960; Powell and Nickerson 1965*a*; Dew 1990). These dense aggregations, or pods, may be composed of several hundred to thousands of individuals.

Podding red king crab in the Kodiak Island area ranged between 17 and 69 mm carapace length (CL) (Powell and Nickerson 1965a; Dew 1990). Juvenile red king crab initially form pods during their second year and continue to display this highly structured behavior as 4-yr-olds. Although Bright (1967) suggested that pods may comprise mixed age-classes, Powell and Nickerson (1965a) reported that age-classes do not intermingle and probably occupy distinct niches.

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The distribution of 4- and 5-yr-old red king crab is poorly known. Aggregations of these age-classes have not been observed in shallow water, and individuals are infrequently caught at depth by conventional sampling methods. Powell and Nickerson (1965a) proposed that these age-classes intermingle and probably inhabit intermediate depths (18–30 m). Vinogradov (1969), however, reported that these age-classes have a similar distribution to adults along the western Kamchatka shelf.

Red king crab attain sexual maturity at 5–6 yr of age over most of their geographic range (Bright et al. 1960; Weber 1965), after which time they undertake seasonal onshore–offshore migrations thought to be directly associated with reproduction and feeding, respectively (Wallace et al. 1949; Bright 1967; Stone et al. 1992). Sexes are believed to be segregated during these migrations and to form discrete groups, separated by age and sex, during much of the year (Wallace et al. 1949; Bright 1967), although supporting data are either weak or nonexistent. Adult red king crab are thought to display gregarious behavior similar to that of early age-classes, but podding behavior in adult crab has been reported only twice (Powell and Nickerson 1965a; Jewett and Powell 1981).[2] These accounts suggest that males and females independently form aggregations preceding mating in shallow-water areas. Early commercial and scientific observations suggest that adult red king crab are generally not uniformly distributed, but rather exhibit a somewhat contagious distribution (Wallace et al. 1949).

The red king crab fishery was one of the most valuable on record until drastic declines in population levels resulted in a complete closure of the fishery in 1983 (Blau 1986; Otto 1986). Although the high resource value provided much incentive, the aggregated distribution of red king crab may have contributed to the high exploitation rates that helped make this fishery so lucrative.

Deductions concerning the spatial pattern of red king crab are based on sampling techniques (i.e., trawl and pot surveys) that introduce biased selectivity (Miller 1990) and lack the capability of discriminating fine-scale details of spatial patterns. Hsu (1987) reviewed the results of the groundfish trawl surveys conducted by the National Marine Fisheries Service (NMFS) between 1972 and 1985 in the southeastern Bering Sea and concluded that females have a highly aggregated distribution at a very large spatial scale. These surveys were conducted over a 6-wk period in June and July each year, over vast areas, with single samples from 400-mi^2 quadrats. Short-term, low-density, random sampling designs probably cannot adequately describe the seasonal distribution pattern of a contagiously distributed species (Dew 1991).

Here we present results of a study to determine the seasonal dispersion of females within one population of red king crab in Southeast Alaska. We document aggregations of adult red king crab and relate this behavior to season, habitat, and life-history events.

[2]Powell and Nickerson (1965a) referred to dense groups of adult red king crab as aggregations, aggregates, or piles. Jewett and Powell (1981) distinguished adult piles or aggregates from juvenile "pods" and described some differences between them. Here we refer to structurally dense and socially organized groups of adults as aggregations. We refer to them as pods if the majority of individuals are in physical contact with each other and stacked atop one another. We refer to the behavior giving rise to these concentrations of adults as aggregating and podding behavior, respectively.

Materials and Methods

This study was conducted in the vicinity of Auke Bay, located 19 km northwest of Juneau, Alaska (Fig. 1). Auke Bay is a small (13 km^2) estuary that supports a local sport fishery for red king crab; commercial crabbing is prohibited in the bay and has been closed in all of southeastern Alaska since 1984. Red king crab are also common in the waters near Auke Bay. Although 45% of the bottom is uniform, muddy, and between 40 and 60 m deep, several submarine trenches and shallow-water reefs associated with seamounts and small islands provide a diversity of habitat (Fig. 1). The physical and chemical oceanography of the study area was described in detail by Bruce et al. (1977).

Ultrasonic transmitters with a 2-yr life expectancy were attached to 10 crab from each of two distinct reproductive classes: primiparous females, or those reproducing for the first time, and multiparous females that had spawned at least once previously. The two reproductive classes are distinguished only by their relative sizes once egg extrusion has occurred; primiparous females have CL $<$ 113 mm (Gray 1963; Vinogradov 1969). Primiparous females were collected at depths between 40 and 50 m with baited pots after they had molted and mated presumably in shallower water. Multiparous females were collected by SCUBA divers as clasping pairs loosely aggregated at depths less than 12 m near the head of the bay. Two additional crab were tagged during the study when derelict tags (i.e., tags which became detached from crab) were recovered by divers. Stone et al. (1992) described tag specifications and tagging methods used in the present study; laboratory and field observations confirmed that tagged crab did not show signs of stress or modified behavior when tagged with these methods.

Primiparous and multiparous crabs were released on 17 May and 3 June 1988, respectively, in areas close to the sites where they were initially captured. The positions of the crab were usually determined weekly, as weather permitted, with an ultrasonic receiver (Sonotronics model USR-4D) and directional hydrophone (Sonotronics model DH-2). A small float was deployed after the boat was positioned over a crab. The boat was maintained at this float while its position was fixed by measuring angles between three shoreward markers with a beam converging sextant used as a pelorus (O'Clair et al. 1990). A total of 28 shoreward markers were used as sextant targets. The relative position of each marker was surveyed with a Wild T-1 theodolite and an electronic distance measuring (EDM) instrument (Keuffel and Esser Co., model AutoRanger II). The minimum between-tag distance for which separate tagged crab positions could be determined was 10 m. During periods when less than 10 m separated the tags, i.e., when crab were tightly aggregated, a general position was recorded for the group of crab, and tagged crab were randomly assigned positions within a 10-m radius from this general position. Depth was measured with a Hummingbird model 4080D depth finder and is reported as metres relative to mean lower low water. Temperature and salinity profiles were measured in the vicinity of tagged crab with a Seabird Electronics Seacat Profiler.

The movements and distribution of seven primiparous and five multiparous females tagged and released in spring 1988 were monitored through one complete reproductive cycle (11–12 mo). The remaining 10 crab tagged in 1988–89 were tracked between 18 and 300 d after release. In spring 1989, two primiparous and four multiparous females were retagged. Twenty additional crab were tagged between 26 May and 7 June: the

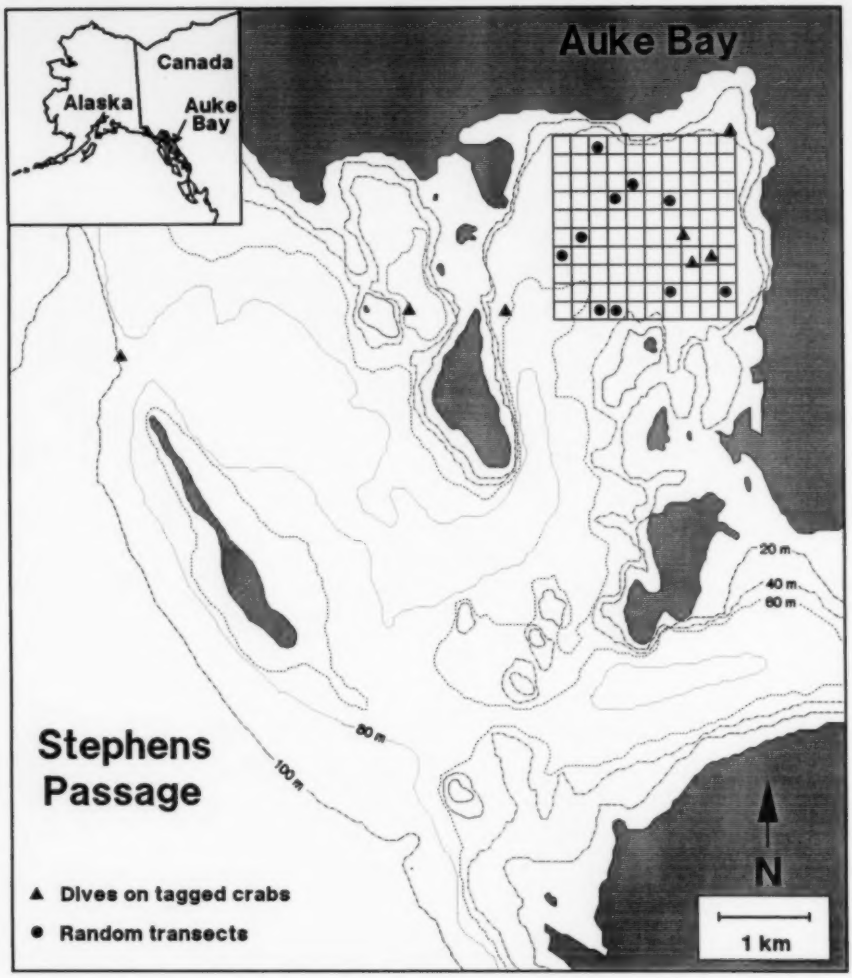

FIG. 1. Study area at Auke Bay showing 10 × 10 sampling grid; circles indicate sites where dives were made on random transects, and triangles denote sites where dives were made on tagged crab. Dives were made in the research submersible *Delta*. Depth contours are in metres.

mates of the four retagged multiparous females, two clasping pairs, six single males, and six single females. All crab were released close to the area of capture. These crab were tagged primarily as subjects for the submersible study described below; their movements and distribution were only casually monitored after that study due to time constraints.

SCUBA was used to make in situ observations of tagged crab when they were within diving depth (<35 m) and to recover molting tagged crab during spring. Divers used a submersible receiver/hydrophone (Sonotronics model USR-88) to locate tagged crab underwater. Divers recorded observations including number of crab sighted, density and structure of aggregations, sex and size composition, depth, activity (i.e., feeding, molting, mating), and habitat characteristics including substrate composition, bottom topography, and biota.

Between 11 and 15 June 1989, 24 dives were made in the two-person research submersible *Delta*. The 4.6-m submersible has 19 viewports and an operating depth of about 350 m. *Delta* was generally operated in direct contact with the sea bottom but did not seem to affect the behavior of any observed king crab. Ten quadrats were randomly selected from a 10 × 10 grid superimposed over inner Auke Bay (Fig. 1), where tagged red king crab were concentrated below 40 m during June 1988. Each quadrat measured 200 × 200 m (area = 40 000 m^2). The submersible was directed along a transect of predetermined bearing also selected randomly in each quadrat. These transects were estimated to be 150 m long (300 s at an average speed of 0.5 m/s). Observations were visually recorded from both sides of the submersible and videotaped by the observer.

Seven dives were also made in the vicinity of tagged crab released in spring 1989 (Fig. 1). A submersible, directional hydrophone was affixed to the compass staff on the bow and the pilot directed the submersible towards a tagged crab by steering in the direction of greatest signal strength. Approximately 20 min was allotted in search of each tagged crab, after which time, crab would be enumerated along a random transect

as above. Four additional dives were made to survey and characterize deepwater habitat where crab from the 1988–89 study had been concentrated.

Weekly positions of tagged crab were plotted with a microcomputer design and drafting program (Autodesk, Inc. 1988). The distance between each tagged crab within inner Auke Bay and its nearest tagged neighbor was measured. A one-way analysis of variance with 1000 random permutations (Edgington 1987) was used to determine if the mean nearest neighbor distance changed seasonally. Fisher's modified least significant difference procedure was used to determine significance of post hoc comparisons (Edgington 1987).

Tagged crab further than 500 m from their nearest tagged neighbor were excluded from the analysis (30 of 34 wk during the 1988–89 tracking study). We excluded these crab from analysis to gain more detailed insights into the aggregation dynamics of one "association" of crab. Up to four outliers were excluded weekly based on this criterion (15.4% of the total number of observations), and the number of weekly outliers showed no apparent seasonal trend. Our observations indicated that the movement and other behavior of crab greater than 500 m from their nearest tagged neighbor were not coordinated with tagged crab from known (via SCUBA and telemetry) aggregations. Additionally, the two dense aggregations we monitored closely during winter were always separated by distances greater than 500 m (based on nearest neighbor distances of tagged crab). No exchange of tagged crab occurred between aggregations separated by 500 m or more whereas the movements of tagged crab within a 500-m radius were often coordinated and directional.

Results

Except for a brief period following release, tagged female red king crab tended to aggregate in relatively small areas of Auke Bay. Although individual crabs often made treks for short distances within discrete areas, large-scale movements to different areas or depths were generally undertaken as a group. Seasonal patterns of movements of the tagged crab are summarized in Stone et al. (1992).

Three seasons were delimited for this study based on crab movements and physical oceanographic cycles as follows: (1) a summer–fall period from June through mid-November, when crab inhabited deep waters ($\bar{x}$ = 52.6 m) below a well-developed thermocline, (2) a winter period from mid-November through early March, when crab inhabited shallow waters ($\bar{x}$ = 27.5 m) and when the water column was well mixed and essentially homogeneous with respect to temperature and salinity, and (3) a spring period from early March through late May marked first by crab movement to intermediate depths ($\bar{x}$ = 33.5 m) and then by movement into shallow water to molt and mate in April–May. This period was also characterized by the redevelopment of the thermocline and halocline during late March.

Female red king crab in this study had distinct seasonal shifts in habitat use (Fig. 2). All tagged females had similar patterns of seasonal habitat use regardless of reproductive class (i.e., primiparous or multiparous). This shift in seasonal habitat use was accompanied by a corresponding shift in the degree of crab aggregation. Female red king crab tended to be more aggregated (mean nearest neighbor distances) in winter than in summer and fall (F-test, $P < 0.01$) (Fig. 3). Females were not ($P > 0.05$) more aggregated in winter than in spring, nor were

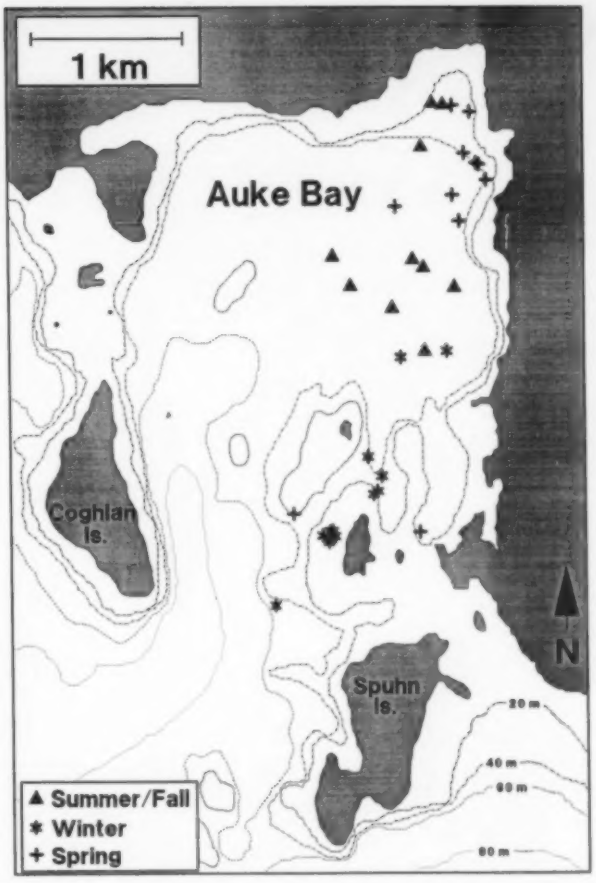

FIG. 2. Map of study area showing the distribution of all tagged female red king crab within Auke Bay during a typical week from each of three seasons. Summer–fall is represented by positions fixed between 23 and 25 August 1988. Winter and spring are represented by fixes on 12 and 13 December 1988 and 30 March 1989, respectively.

there any differences between summer–fall and spring dispersion patterns (Fig. 3). Mean water temperature at depths occupied by tagged crab was not correlated ($P > 0.50$, Spearman rank, $r_s = 0.11$) with mean nearest neighbor distances, despite the apparent trend between the two variables seasonally (Fig. 3). Depth of tagged crab was significantly correlated ($r^2 = 0.30$, $N = 34$, $P < 0.01$) with mean nearest neighbor distances (Fig. 3).

During winter, tagged females were tightly aggregated, and individual positions could not be determined for these crab on seven occasions. The number of tagged crab occupying the same position ranged from two to seven, and the same individuals often remained together for extended periods (i.e., four crabs remained within the same group between 18 November 1988 and 13 January 1989). This situation also occurred four times during summer and three times during spring, but only once involved more than two crab. These crab, however, never shared the same position during consecutive weeks.

Diver observations during winter revealed that tagged females were often associated with dense aggregations and sometimes pods estimated by in situ counts to consist of 300 or more indi-

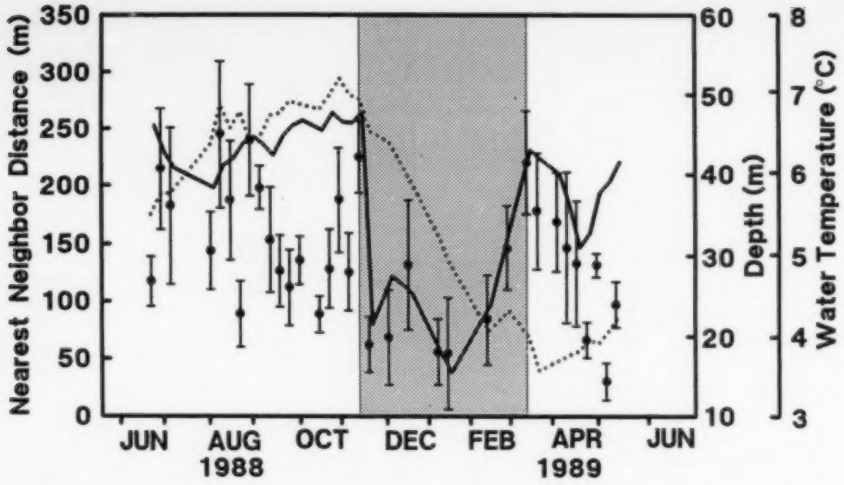

FIG. 3. Mean nearest neighbor distance (±1 SE of the mean) for all tagged crab within inner Auke Bay (outliers were removed). Mean depths (solid line) and mean water temperature at depths (dotted line) occupied by tagged crab are also graphed. The shaded area represents the period between fall and spring thermohaline mixing.

FIG. 4. Aggregation of approximately 150 ovigerous female red king crab at 18 m depth in mid-January 1990. The aggregation has begun to disband after being disturbed by divers. (Photograph by J. Lincoln Freese)

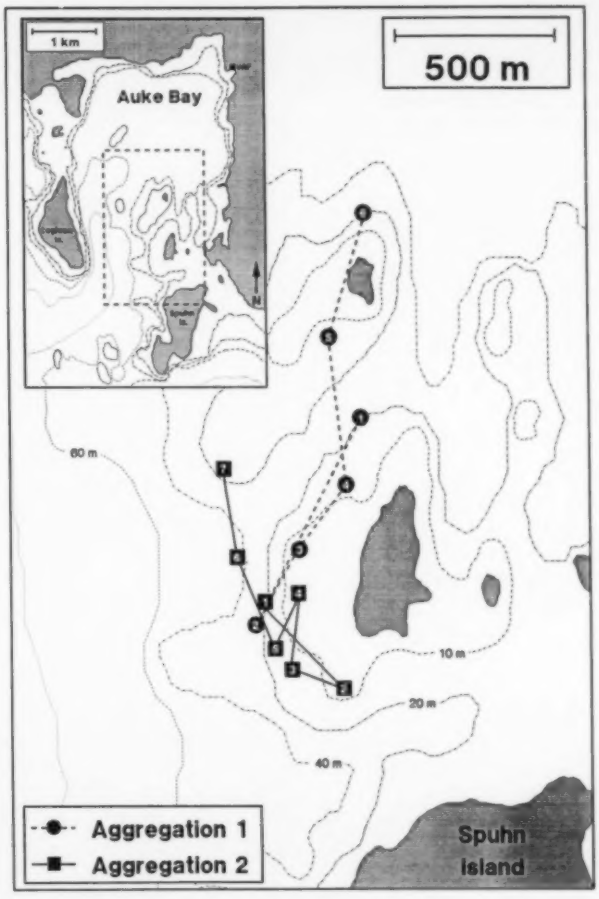

FIG. 5. Movements of two aggregations of female red king crab tracked between 18 November 1988 and 30 March 1989 in Auke Bay. Symbols mark weekly locations; numbers denote week of study period.

viduals (Fig. 4). Most crab in the vicinity of an aggregation were closely associated with it, as was evident by the small number of crab found as divers swam away from an aggregation. Movement patterns for two independent aggregations tracked for 81 and 77 d during this period are detailed in Fig. 5. Aggregation 1 was considered to be a stable aggregation on the basis that between four and eight tagged crab remained together for a period of several months. In addition, biweekly diver observations during this period indicated that the tagged crab were always associated with several hundred crab. Although aggregation 2 consisted of only one tagged crab, we observed that this crab was part of a large aggregation between 12 January 1989 and 30 March 1989. During this period, diver observations confirmed that this crab was always found in the presence of several hundred other crabs. The tagged crab (and presumably the same association of crab) occupied the same general area and displayed very limited movements during this period (Fig. 5).

Although movement patterns and habitat use were remarkably similar for these two aggregations, they were temporally and spatially separated ($\bar{x}$ = 925 m) and were considered to be distinct aggregations on that basis. In addition, no exchange of tagged crab between the two groups was observed. Aggregation 1 was first observed on 18 November 1988 and was tracked until 7 February 1989, when crab apparently disbanded somewhat and moved to intermediate depths. This aggregation moved a total distance of 2169 m, had a mean movement rate of 27 m/d, and occupied shallow areas with a mean depth of 15.5 m. Aggregation 2 was first observed on 12 January 1989 and was monitored through 30 March 1989, after moving a total distance of 1695 m. This group of crab had a mean movement rate of 22 m/d and a mean depth of 19.1 m. Total displacements (straight-line distance from first to last positions) for these aggregations were 710 and 495 m, respectively. Water temperatures at depths occupied by these aggregations steadily decreased and ranged from 6.4 to 3.9°C and from 4.8 to 3.7°C, respectively (Table 1). Salinities at these same depths steadily increased and ranged from 29.5 to 31.3‰ and from 30.7 to 32.1‰, respectively (Table 1). Weekly tracking records for the aggregations are presented in Table 1.

Adult pods were not structurally similar to pods of juveniles. Pods were typically asymmetrical, and crab were usually stacked only three or four individuals deep (Fig. 6). Pods were often closely associated with shallow-water reefs and sometimes stacked up against them. These reefs were typically bedrock outcrops, surrounded by silt–sand slopes, and were covered with macrophytes and sessile invertebrates. When not exhibiting podding behavior, females were generally inactive with their walking legs and abdomens partially buried in the silt–sand bottom. We occasionally observed crab actively feeding on *Pycnopodia helianthoides*, *Evasterias troschelii*, *Strongylocentrotus droebachiensis*, and *Balanus* spp.

Aggregations in shallow water (mean depth <25 m) were composed of both primiparous and multiparous females, although in situ diver counts indicated that multiparous females always predominated. We observed males with these shallow-water aggregations as early as mid-January, but mating was not observed prior to 1 March 1989. Clasping pairs consisting of large, legal-sized (CL > 145 mm) males and virgin females were observed near the periphery of these aggregations into early March, after which time, most females had migrated to intermediate depths inaccessible to divers. Virgin females were identified by their small size and lack of egg remnants on their pleopodal setae. A few tagged crab remained in shallow-water areas, however, and diver observations confirmed that females continue to form dense aggregations through late March. Although large males were often found near these aggregations, no molting or mating was observed. Dense aggregations of crab were not observed after 31 March, and diver observations suggested that females generally dispersed into loose aggregations of up to 60 crab until molting and mating.

Near-bottom visibility from the manned submersible *Delta* was often less than 1 m and likely contributed to the limited number of red king crab observations in deepwater habitats. No red king crab were observed along transects in randomly chosen quadrats in Auke Bay (Fig. 1); however, Tanner crab (*Chionoecetes bairdi*) were observed along 6 of 10 transects. Only 13 red king crab were sighted from the submersible; all of these sightings were in the immediate vicinity of tagged crab. Three tagged red king crab were located from the research submersible, and untagged red king crab and Tanner crab were observed in the vicinity of these tagged crab. Although attempts to locate and observe four other tagged crab from the research submersible failed, untagged red king crab were observed in the vicinity of two of those crab.

TABLE 1. Tracking records for two aggregations of female red king crab monitored in Auke Bay. Weekly locations of each aggregation are shown in Fig. 5.

Week No.	Date	Distance moved (m)	Depth (m)	Temp. (°C)	Salinity (‰)	No. of tagged crabs
		Aggregation 1				
1	18 Nov. 1988	—	18.6	6.4	29.5	4
2	2 Dec. 1988	735	23.5	6.3	29.8	4
3	13 Dec. 1988	283	8.5	5.5	29.3	5
4	6 Jan. 1989	255	11.0	5.1	30.3	7
5	13 Jan. 1989	478	11.6	4.8	30.7	8[a]
6	7 Feb. 1989	417	19.5	3.9	31.3	7[b]
		Aggregation 2				
1	12 Jan. 1989	—	15.9	4.8	30.7	1
2	26 Jan. 1989	369	9.5	3.2	30.5	1
3	7 Feb. 1989	199	11.3	3.7	31.2	1
4	22 Feb. 1989	260	8.5	4.1	31.5	1
5	6 Mar. 1989	225	19.5	3.8	31.8	1
6	14 Mar. 1989	328	53.0	3.6	32.1	1
7	30 Mar. 1989	314	16.2	3.7	31.7	1

[a]Includes one tagged crab 30 m from a dense aggregation of seven tagged crab.
[b]Includes one tagged crab (same individual as above) 40 m from a dense aggregation of six tagged crab.

FIG. 6. Pod of ovigerous female red king crab in shallow water (≈17 m depth). Females were observed occasionally stacked three or four atop each other. A large legal-sized male (CL > 145 mm) is visible at the right edge of the pod. (Photograph by J. Lincoln Freese)

The distribution of crab tagged and released in May and June 1989 was monitored only periodically, yet the patterns of seasonal distribution for these crab and those monitored weekly from May 1988 to May 1989 were remarkably similar. Furthermore, SCUBA observations confirmed that tagged crab formed dense aggregations and exhibited podding behavior during both years and that there was little interannual variation in the areas where these aggregations formed.

Discussion

We observed two levels of dispersion of female red king crab during this study: (1) loosely knit aggregations in deep water (>40 m) during the summer and fall and (2) dense aggregations and pods in shallow water (<25 m) during winter and early spring. The factors causing the two observed levels of dispersion may be depth specific. Habitat heterogeneity is an obvious cause of aggregation in some species, especially mobile ones (Brown and Orians 1970; Poole 1974). Species tend to aggregate in areas providing adequate resources and suitable conditions. Water temperature and salinity did not differ significantly at adjacent areas of both the deepwater (>40 m) and shallow-water (<25 m) habitats, yet tagged crab utilized only a small percentage of the available habitat during all seasons (Stone et al. 1992). Crab aggregated within well-defined areas, while adjacent, seemingly identical habitats were seldom utilized. These observations may indicate that resources were patchily distributed within habitats. Although the dispersion patterns we observed in deep water may be explained by resource patchiness and habitat heterogeneity alone, other behavioral, or possibly physiological, mechanisms are probably involved in the formation of dense aggregations and pods in shallow water.

If the clumping of individuals is greater than would be expected on the basis of resource patchiness alone, the distribution of individuals (home range) may be influenced by the presence of conspecifics (Brown and Orians 1970). Excluding reproductive aggregations and aggregations near clumped resources, Brown and Orians (1970) identified four functions that could be enhanced by associations of individuals: (1) group defense against predators, (2) group defense of feeding areas, (3) the ability to exploit resources more readily than solitary individuals, and (4) the ability to profit from the foraging success of other individuals by observing where they find food. Although detailed in situ observations of red king crab feeding behavior are lacking, they are considered to be opportunistic predators/scavengers (Jewett and Feder 1982). As such, their foraging success is probably not significantly enhanced by group behavior. The last three functions listed by Brown and Orians (1970) may be enhanced by group association, but none is likely to be the primary cause of aggregating behavior in red king crab. Group defense against predators (i.e., earlier predator detection via increased vigilance of larger groups (Magurran 1990)), however, may be one function of podding behavior in red king crab.

Predator defense is most often proposed as the cause of podding behavior in juvenile red king crab. Juvenile red king crab are thought to form pods mainly as protection against predators (Powell and Nickerson 1965a; Powell 1972; Somerton 1985). Dew (1990) suggested that juveniles begin to pod as they outgrow available refuges. He further suggested podding in juveniles as a strategy to avoid detection by a diurnally active, randomly searching predator; podding is more typical during the day. Podding behavior might be an alternative strategy to refuge seeking behavior for growing crab that must increase their foraging efforts into daylight hours, and hence increase their vulnerability to predation (Dew 1990). The "selfish herd" hypothesis (Hamilton 1971) states that individuals might increase their fitness by joining conspecifics in dense aggregations. According to this hypothesis, gregarious behavior is a selfish cover-seeking instinct of individuals that attempt to put conspecifics between themselves and potential predators (Hamilton 1971).

Whether podding also serves as a behavioral defense mechanism in adult red king crab is unknown. The giant Pacific octopus, *Octopus dofleini*, may be an important shallow-water predator of adult red king crab. An ultrasonic tag attached to a primiparous female in spring 1989 was found at a depth of 15 m in the entrance to an occupied octopus den. Predators that forage at relatively shallow depths (i.e., sea otters, *Enhydra lutris*) may have been important in shaping adult podding behavior on an evolutionary time scale. However, the role of these predators in influencing red king crab behavior remains to be demonstrated.

Podding behavior has also been reported for several other large adult crustaceans. Dense aggregations of the spiny lobster, *Panulirus argus*, have been observed to form for the purposes of migrating (Berrill 1975). Campbell (1990) attributed the clumped distribution of ovigerous female American lobsters, *Homarus americanus*, to selection of preferred habitats for egg development and larval dispersal. Podding behavior has been noted in four species of majid crabs and has been suggested as a protective mechanism during molting and mating (Carlisle 1957; Števčić 1971; DeGoursey and Stewart 1985; Hanauer 1988; Stevens et al. 1992).

Dense aggregations of adult red king crab have only been reported during late winter and early spring and are also thought to form strictly for the purpose of molting and mating (Powell and Nickerson 1965b). Female red king crab in this study began to form dense aggregations as early as November after migrating to shallow water. Male crab, however, were not observed in association with these aggregations until late January, and molting and mating were not observed until early March. Although some degree of aggregation certainly exists after March, when crab seemed to disband somewhat and migrate to intermediate depths, clasping pairs were generally solitary in shallow-water areas. Clasping pairs often utilized macrophytes and reef complexes as cover, but only remained in shallow-water areas for a brief period following mating. Molting and mating were occasionally observed near aggregations, but only near the periphery, never within aggregations, as has been reported for other species (Carlisle 1957; Števčić 1971; DeGoursey and Stewart 1985; Hanauer 1988; Stevens et al. 1992). Formation of dense aggregations would concentrate females near spawning areas, increase the frequency of male–female encounters, and consequently improve mating opportunities. A similar purpose for shallow-water aggregations of Dungeness crab, *Cancer magister*, has been suggested by Butler (1960).

Whether or not adults form dense aggregations or pods at greater depths (>40 m) remains unresolved, although analysis of mean nearest neighbor distances would indicate that this behavior is more typically a shallow-water phenomenon associated with rapidly decreasing water temperatures there (Fig. 3). Powell (1974) suggested that adults probably do not form pods at depth because of the increased amount of time they spend

foraging during adulthood. This behavior could be related to prey distribution and abundance patterns. Dew (1990) attributed the daily podding behavior and foraging dynamics of juvenile red king crab to changes in water temperature (i.e., they spent more time foraging as unpodded crab as water temperature increased). Although podding behavior in adult females may also be associated with decreased water temperatures during winter when females are less active (Stone et al. 1992) and food consumption is decreased (Jewett and Feder 1982), we did not find a significant correlation between nearest neighbor distance (a measure of the degree of podding behavior) and temperature. The controlling mechanisms for podding behavior in adult females may be quite different from those in juveniles, especially during early spring when females prepare to molt and mate.

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Effects of Early Rearing Environment on Stress Response, Immune Function, and Disease Resistance in Juvenile Coho (*Oncorhynchus kisutch*) and Chinook Salmon (*O. tshawytscha*)

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Coho (*Oncorhynchus kisutch*) and chinook salmon (*0. tshawytscha*) from aquaculture and wild environments were subjected to handling (30–60 s of netting and aerial emersion) and disease challenges. Plasma cortisol concentrations ($[cortisol]_{pl}$) in both coho and chinook salmon from wild environments were significantly elevated 4 h after handling. Colonized coho salmon (hatchery-reared fish, transported into a natural water body as fry) responded in a similar fashion to wild fish, while those reared entirely in the hatchery showed no significant rise in $[cortisol]_{pl}$. The responses to handling stress were retained in wild and colonized coho salmon after 7 mo of hatchery rearing. A transient increase in the leukocyte to red blood cell ratio in both wild and hatchery-reared chinook salmon occurred 4 h after handling. Handling signficantly decreased the antibody-producing cell (APC) number in wild fish and elevated their $[cortisol]_{pl}$ relative to hatchery fish. Wild fish had the highest APC number among the three groups before the handling. No difference in resistance to *Vibrio anguillarum* was apparent in coho and chinook salmon among the different rearing environments, although chinook salmon were generally more susceptible; disease resistance was reduced in wild coho salmon after 7 mo of rearing in a hatchery.

Des saumons cohos (*Oncorhynchus kisutch*) et quinnats (*O. tshawytscha*) d'élevage et sauvages ont été soumis à une manutention (sortie de l'eau à l'épuisette de 30 à 60 s) et à une mise en présence d'un pathogène. Les concentrations plasmatiques de cortisol ($[cortisol]_{pl}$) des saumons sauvages des deux espèces demeuraient élevées de façon significative 4 h après la manutention. Des saumons cohos ensemencés (poissons d'élevage ensemencés dans un plan d'eau naturel à l'état d'alevins) ont réagi de façon semblable aux poissons sauvages tandis que ceux dont la croissance avait été totalement faite en pisciculture n'ont pas présenté une telle élévation du $[cortisol]_{pl}$. La réponse au stress de la manutention des saumons cohos sauvages et ensemencés était encore perçue après 7 mo en pisciculture. Une augmentation transitoire du rapport entre les leucocytes et les érythrocytes a été notée chez les saumons quinnats sauvages et d'élevage 4 h après la manutention. Celle-ci a eu pour effet, comparativement aux poissons d'élevage, d'abaisser de façon significative le nombre de cellules productrices d'anticorps (CPA) chez les poissons sauvages en plus d'élever la $[cortisol]_{pl}$. Les poissons sauvages sont ceux des trois groupes qui présentaient le nombre le plus élevé de CPA avant la manutention. Aucun écart de la résistance à *Vribio anguillarum* n'a été noté chez les cohos et les quinnats provenant des deux types de milieu de croissance, mais les quinnats étaient, de façon générale, plus sensibles; la résistance à la maladie était encore réduite chez les cohos sauvages après 7 mo en conditions d'élevage.

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Intensive aquaculture is used both as a source of food and as a tool in fisheries management. The release of large numbers of intensively cultured juveniles to supplement wild fish stocks is a common practice in North America. In spite of these stocking efforts, survival rates (smolt to adult) of coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) hatchery-reared smolts have declined in the Pacific northwest over the past decade (McIntyre 1985). Survival of wild coho salmon is reported to be 1.5–2.0 times higher than that of their hatchery-reared counterparts (Felton et al. 1990). A similar comparison with chinook salmon in the Lewis River, Washington (a tributary of the Columbia River), found that wild fish had average survival rates 2.6 times higher than those of hatchery-reared fish (Felton et al. 1990). Furthemore, Reisenbichler (1988) found that salmon stocks transferred into environments other than those in which they were locally adapted had poorer return rates than indigenous populations, indicating that a greater similarity between point of origin and transfer location improved survival. Reisenbichler (1988) also found that wild fish from different river systems were more similar to each other than a wild stock and its hatchery counterpart from the same river system.

Rearing conditions within hatcheries may also affect later survival. Fagerlund et al. (1981) found that return rates of adult coho salmon were inversely proportional to their hatchery rearing density. Furthermore, Hosmer et al. (1979) found that

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Atlantic salmon (*Salmo salar*) reared in water with reduced flow rates had significantly lower rates of returning adults.

Fish are more susceptible to disease when they are stressed (Wedemeyer et al. 1984). During stress, interrenal cells in the anterior kidney hypertrophy and increase cortisol secretion (Patiño et al. 1987). Cortisol is the primary corticosteroid of teleost fish (for reviews, see Patiño et al. 1987; Barton and Iwama 1991), and elevated levels in plasma have been shown to be immunosuppressive (McLeay 1975; Ellsaesser and Clem 1986; Maule et al. 1987; Tripp et al. 1987). A high physiological plasma cortisol concentration ($[cortisol]_{pl}$) is associated with a decrease in the levels of circulating lymphocytes (McLeay 1975; Barton et al. 1987) and changes to hematopoietic tissues and cells (Peters and Schwarzer 1985). Acute stress has been shown to cause a reduction in the number of antibody-producing cells (APC) as early as 4 h after the disturbance (Maule et al. 1989). An elevation of the $[cortisol]_{pl}$ has been shown to increase the susceptibility of some salmonids to infectious diseases (Wedemeyer et al. 1984; Pickering and Pottinger 1989), possibly through cortisol-mediated immunosuppression (Maule et al. 1987; Tripp et al. 1987).

Differences between wild and hatchery-reared fish have been observed with respect to their morphology (Hjort and Schreck 1982), behaviour (Dickson and MacCrimmon 1982; Smith 1985), and physiology (Woodward and Strange 1987; Iwama et al. 1992). The stress response of wild and hatchery-reared fish to handling has been examined and a heightened cortisol response has been found in fish of wild origin (Woodward and Strange 1987; Iwama et al. 1992).

The objective of this study was to examine possible physiological differences between fish of similar genetic makeup reared in different early rearing environments. The measured parameters included the stress response after handling, as determined by changes in $[cortisol]_{pl}$, resistance to the bacterial disease vibriosis, and immunological parameters thought to be important to disease resistance. These were examined in groups of coho and chinook salmon that had different rearing histories in the early life stages. Wild chinook salmon and their hatchery-reared counterparts were studied. In addition to these two rearing histories in coho salmon, a group that was reared in the hatchery and transferred to a natural water body as fry, termed the colonized group, was studied. Furthermore, the above parameters were reexamined in the three coho salmon groups after 6 mo of further rearing in a hatchery environment to determine if any physiological differences among the groups would be maintained over time, or whether acclimation to such an environment would occur in particularly the wild and colonized groups.

Materials and Methods

Fish

The hatchery, wild, and colonized groups discussed below were all juvenile salmon hatched from eggs of returning adult spawners to those river systems. The wild fish developed from eggs spawned naturally in the river beds. The hatchery fish were artificially propagated from eggs and milt taken from the same group of spawners and maintained for the entire rearing period in a hatchery environment until the time of their release. Colonized fish were those fish of similar hatchery origin that were released 5 mo post-hatch into the upper watershed of the river as fry. The naturally reared coho and chinook salmon were assumed to be genetically similar to their hatchery counterparts. Any physiological differences observed were interpreted as being due to differences in physical rearing environments and the selective pressures of those environments. At the time of sampling in the field, the majority of fish in all groups exhibited silver colouring, indicative of fish that had undergone smoltification. Comparisons between groups were made of fish in the same physiological state, but not necessarily of the same age or size.

On May 16, 1990, juvenile wild chinook salmon smolts were netted from the Big Qualicum River on Vancouver Island, British Columbia (B.C.), Canada, during their nocturnal migration as they passed through open gates in a fish counting fence. The following day, their hatchery counterparts were arbitrarily sampled from the concrete raceways of the big Qualicum River Fish Hatchery, Department of Fisheries and Oceans (DFO), Qualicum Beach, B.C., just prior to their timed release as smolts. The mean weights (± 1 SE) at capture were 3.2 ± 0.1 g for wild smolts and 6.1 ± 0.2 g for hatchery smolts. On May 26, 1990, juvenile wild and colonized coho salmon smolts and their hatchery counterparts were sampled as described above from the Quinsam River and Quinsam River Fish Hatchery (DFO), near Campbell River, B.C. Colonized smolts were distinguished from other migrants by size and adipose fin clips, indicating their hatchery origin. The mean weights (± 1 SE) at capture were 8.7 ± 0.5 g for wild smolts, 17.5 ± 0.8 g for colonized smolts, and 24.1 ± 1.0 g for hatchery smolts. Of these fish, approximately 1000 each of wild and hatchery chinook salmon, and 1000 each of wild, colonized, and hatchery coho salmon were brought to the Aquaculture Unit of the Department of Animal Science at the University of British Columbia (UBC). Before the first set of experiments, these groups were held separately in 1000-L oval tanks continuously supplied with dechlorinated Vancouver tap water (7–8°C, pH 6.1, total hardness 4.2 mg/L, $[O_2]$ 10 ppm). Fish were fed a maintenance diet of EWOS grower pellets (EWOS Canada Ltd., Surrey, B.C.) at 1–2% body weight daily. Wild fish were initially fed freeze-dried krill (Argent Laboratories, Richmond, B.C.) and gradually introduced to pellets over a period of 2 wk. All groups were subject to the same husbandry practices, consistent with normal culture at the facility. The fish were subjected to the experimental procedures described below after a 1-mo acclimation period to the holding facility at UBC.

The stress responses in hatchery, wild, and colonized coho salmon were determined by the measurement of $[cortisol]_{pl}$ in response to a handling stress, as described below. The same set of experiments were conducted with hatchery and wild chinook salmon juveniles. Disease resistance to *Vibrio anguillarum*, a causative agent of vibriosis, was determined for all groups of fish.

In addition to the above experiments which were conducted after an acclimation period of 1 mo, the handling stress and disease challenge experiments were conducted with juvenile coho salmon from the original stocks of wild, hatchery, and colonized groups after they were reared at the UBC Aquaculture Unit for a further 6 mo; this brought their total rearing time there to 7 mo. At the end of that time, the possibility that acclimation of particularly the wild and colonized groups to the rearing conditions in the hatchery was studied by measuring the responses of the three coho salmon groups to the handling and disease challenges. The $[cortisol]_{pl}$ response and the APC number in the anterior kidney were measured at 4 h after the

handling stress, and disease resistance to the above pathogen was also determined. The protocols for the handling stress and disease challenge were identical to the initial experimemts after 1 mo acclimation. After 7 mo in fresh water, the juvenile coho salmon were considered to have reverted to parr as indicated by the loss of the silver colouration and the presence of parr marks on their bodies.

Handling Stress

In each experiment the fish were acutely stressed by rapidly netting and holding them out of the water for 30–60 s. Each group was then returned to 200-L holding tanks. This approach has been demonstrated to evoke a large rise in $[cortisol]_{pl}$ within a short time in salmonids and other fishes (see Barton and Iwama 1991). Groups of coho salmon were then sampled from their holding tanks at 8 and 30 h following the handling stress. Chinook salmon were sampled at 4, 8, and 30 h following the handling stress in the same manner. The additional 4-h sampling period was added to the sampling schedule for the chinook salmon in case the peak elevation in $[cortisol]_{pl}$ was being missed by sampling at 8 h post-stress. Constraints on time and availability of fish precluded the taking of a 4-h sample of $[cortisol]_{pl}$ from the coho salmon.

At each time of sampling, 12 fish were rapidly netted and transferred to a bucket containing a lethal dose (200 mg/L) of buffered tricaine methanesulfonate (MS222). This dose has been shown to inhibit a further rise in $[cortisol]_{pl}$ in salmon after anaesthetization (Barton et al. 1986). Control samples were taken at 0 h from the stock tanks before the fish were netted for the stress group. After the fish were killed, the caudal peduncle was severed and blood was collected in heparinized capillary tubes. Plasma was separated by centrifugation (model MB microhematocrit centrifuge; International Equipment Co., Massachusetts) and stored at −20°C for later determination of cortisol concentration as described below. Fork length (Ln) and weight (W) were recorded and were used to determine the condition factor (K) of the fish by Pickering and Duston's (1983) equation: $(K = W\,(g)\cdot Ln\,(cm)^{-3}\cdot 100)$ (Table 1).

Hematology

Hematocrit values were determined as percent packed cell volume after centrifugation for 5 min at 11 500 rpm in a microhematocrit centrifuge (model MB; International Equipment Co., Massachusetts). The ratios of total leukocytes, lymphocytes, and thrombocytes to 1000 red blood cells (RBC) were manually determined from Wright–Giemsa-stained blood smears by counting erythrocytes (average 637 per slide, range 475–802), lymphocytes, and other leukocytes in nine random fields per slide (i.e., per fish) using Yasutake and Wales (1983) as a reference for the different cell types and variants.

Plasma Cortisol Assay

The $[cortisol]_{pl}$ was determined by radioimmunoassay [^{125}I] (Clinical Assay No. 529, Baxter Healthcare Corp., Cambridge, MA) based on competitive binding principles (Foster and Dunn 1974).

Specific Immune Response in vitro

To assess the ability of lymphocytes to generate specific antibodies, we established cell cultures of leukocytes as described by Tripp et al. (1987) and Maule et al. (1989). All chemicals and reagents, unless otherwise noted, are from Sigma Chemical Co. (St. Louis, MO). Duplicate 50-μL samples of anterior kidney tissue homogenate per fish (n = 12) containing 2×10^7 leukocytes/mL samples were incubated for 7 d in 96-well microtitre plates (Falcon Microtest III tissue culture plates, Becton Dickinson and Co., Lincoln Park, N.) with 50 μL of tissue culture media (TCM) containing the antigen trinitrophenalated-lipoplysaccharide (TNP-LPS) or TCM only (negative controls). When immunized against TNP-LPS in this manner, the lymphocytes secreting anti-TNP antibody can be detected by the Cunningham plaque assay (Cunningham and Szenberg 1968). At the time of the plaque assay, a 50-μL aliquot of the cell culture suspension in each well was mixed with 10 μL of 10% adult coho salmon serum (the source of complement) in modified barbital buffer and 10 μL of TNP-tagged sheep red blood cells (SRBC). This mixture was put into sealed Cunningham chambers at 17°C for 2 h. The antigen–antibody complexes activate complement-mediated SRBC lysis, causing the appearance of holes or plaques in the lawn of TNP-tagged SRBC where hemolysis occurs, each hole corresponding to an APC. The number of viable leukocytes at the time of the assay was determined using a hemacytometer and trypan blue dye exclusion. Results were expressed as number of APC per million viable leukocytes.

TABLE 1. Mean (± 1 SE) weight, length, and condition factor of juvenile coho (n = 36) and chinook salmon (n = 30) reared for 1 mo in a hatchery environment and of coho salmon after 6 mo in a hatchery environment. For each group, and by column, values with superscripts that are different indicate significant differences ($P \leq 0.05$).

Group	Weight (g)	Length (cm)	Condition factor
	Coho salmon		
Hatchery	22.5 ± 2.0^a	14.0 ± 0.4^a	0.85 ± 0.02^a
Wild	10.4 ± 1.0^b	10.1 ± 0.3^b	0.91 ± 0.02^a
Colonized	18.6 ± 1.4^a	12.5 ± 0.3^c	0.95 ± 0.03^a
	Chinook salmon		
Hatchery	6.9 ± 0.3^a	8.8 ± 0.1^a	1.0 ± 0.01^a
Wild	4.8 ± 0.2^b	7.8 ± 0.1^b	1.0 ± 0.02^a
	Coho salmon (reared in a hatchery environment for 7 mo)		
Hatchery	82.4 ± 9.0^a	19.8 ± 0.7^a	1.01 ± 0.03^a
Wild	37.1 ± 2.9^b	15.2 ± 0.3^b	1.04 ± 0.02^a
Colonized	55.3 ± 7.7^b	17.2 ± 0.8^b	1.02 ± 0.02^a

Disease Challenges

All disease challenges were conducted on fish that did not receive any handling stress. For each species, duplicate treatment groups of 32 fish per group, each representing a different early rearing history, were transferred to 10 separate 200-L oval tanks (coho: 3 rearing histories × 2 tanks/rearing history; chinook: 2 rearing histories × 2 tanks/rearing history) with the same water source as the 1000-L stock tanks and allowed to acclimate to those tanks for 2 wk prior to being challenged. On June 18, 1990, wild and hatchery chinook salmon were exposed to *V. anguillarum* (isolate RI; source: T.P.T. Evelyn, Pacific Biological Station, Nanaimo, B.C.) by the bath immersion method (Gould et al. 1979) at a challenge concentration of

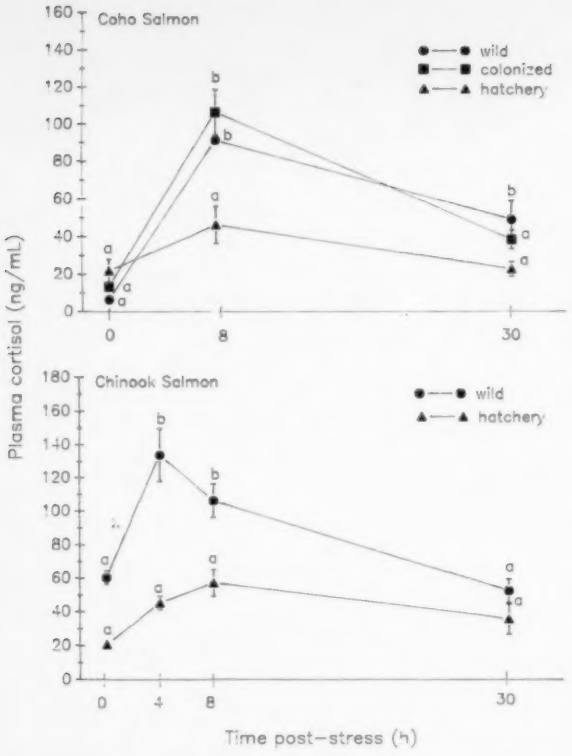

FIG. 1. Mean (±1 SE) $[cortisol]_{pl}$ ($n = 12$) at 0, 4, 8, and 30 h following a 30- to 60-s dipnet handling stress of juvenile coho and chinook salmon from different early rearing environments. Differing superscripts indicate significant differences ($P \leq 0.05$) among means.

1×10^7 colony-forming units (cfu)/mL. On July 3, 1990, wild, hatchery, and colonized coho salmon were similarly exposed to 5×10^6 cfu/mL. Mean water temperature for both challenge experiments was 8°C. On February 6, 1991, approximately 7 mo after the first series of challenges, duplicate treatment groups of 21 coho salmon per group from the original 1000-L stock tanks were similarly transfered and challenged with 1.3×10^6 cfu/mL at a mean water temperature of 9°C. For each fish group within a sample population, a control group of 32 fish was treated similarly, but without the addition of *V. anguillarum* to the immersion bath. Mortalities were removed daily and deaths due to the pathogen were confirmed by isolation of *V. anguillarum* from the kidney on trypticase soy agar (TSA) plates (4% TSA, 0.5% NaCl). Data were expressed as percent total cumulative mortality over 15 d.

Data Analysis

Analysis of variance was applied to the data and where significant differences were found, Tukey's test (Steel and Torrie 1980) was used to identify treatment means that differed significantly. As the mortality in the duplicate treatments of the disease challenges did not differ significantly within groups, the results were pooled and then analyzed using contingency tables (chi-square analysis) to test for independence of the means (Duncan et al. 1983). Statistical significance was determined at the 5% level in all tests.

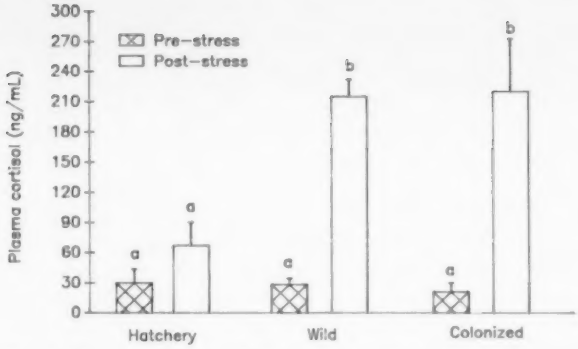

FIG. 2. Mean (±1 SE) $[cortisol]_{pl}$ ($n = 12$) at 4 h following a 30- to 60-s dipnet handling stress of wild, colonized, and hatchery coho salmon held in a hatchery environment for 7 mo. Differing superscripts indicate significant differences ($P \leq 0.05$) among means.

Results

Plasma Cortisol

The $[cortisol]_{pl}$ did not increase significantly above the pre-stress levels at any of the sampling times in the hatchery-reared chinook as well as in the hatchery-reared coho salmon subjected to the handling stress (Fig. 1). Handling consistently produced a significant increase in $[cortisol]_{pl}$ in juvenile wild chinook salmon and wild and colonized coho salmon (Fig. 1). The $[cortisol]_{pl}$ of wild and colonized coho salmon was significantly elevated over the pre-stress level at 8 h after handling (Fig. 1). The $[cortisol]_{pl}$ of wild chinook salmon was significantly elevated over the pre-stress level at both 4 and 8 h after handling (Fig. 1). In all groups, except the wild coho salmon, the 30-h post-stress $[cortisol]_{pl}$ was not different from the control value (Fig. 1).

It appeared that a further 6 mo of rearing in a hatchery rearing enviroment did not change the $[cortisol]_{pl}$ in response to handling at 4 h (Fig. 2). Wild and colonized coho salmon had significantly elevated $[cortisol]_{pl}$ at 4 h, while the hatchery fish showed a small, but insignificant increase in $[cortisol]_{pl}$ over the pre-stress level (Fig. 2).

Hematology

There was a transient increase in lymphocyte and leukocyte counts, relative to RBC numbers, in the 4- and 8-h post-handling sample times of both wild and hatchery chinook salmon (Table 2). At 30 h post-handling, the lymphocyte and leukocyte counts were not significantly different from pre-stress values (Table 2). There were no significant differences between lymphocyte and leukocyte counts relative to RBC at any of the sampling times for both wild and hatchery-reared coho and chinook salmon (Table 2). These ratios were consistently higher at the pre-stress sampling times from wild fish than from their hatchery counterparts. Hematocrit values in both hatchery-reared and wild chinook salmon were not significantly different from the pre-stress hematocrit values at any sampling time (Table 2). No significant differences from the pre-stress values in any of the hematological parameters following handling were observed in the coho salmon.

Following a total of 7 mo in the hatchery environment, no significant changes were evident in most of the hematological

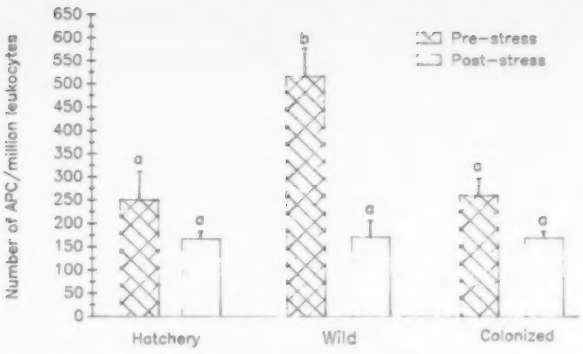

FIG. 3. Mean (± 1 SE) number of APC per million viable leucocytes (n = 12) at 4 h following a 30- to 60-s dipnet handling stress of wild, colonized, and hatchery coho salmon after holding in a hatchery environment for 7 mo. Differing superscripts indicate significant differences ($P \leq 0.05$) among means.

TABLE 2. Mean (± 1 SE) lymphocyte to RBC and total leukocyte to RBC ratios per 1000 RBC and hematocrit (% packed RBC volume) of chinook salmon (n = 12) at 0 (control), 4, 8, and 30 h following a 30- to 60-s dipnet handling stress. Different superscripts indicate significant differences ($P \leq 0.05$).

Time post-stress (h)	Lymphocytes per 1000 RBC	Leukocytes per 1000 RBC	Hematocrit
	Hatchery		
Control	11 ± 2^{a}	12 ± 2^{a}	49 ± 1^{a}
4	20 ± 3^{b}	24 ± 3^{b}	50 ± 1^{a}
8	14 ± 3^{ab}	15 ± 3^{ab}	51 ± 1^{a}
30	11 ± 2^{a}	12 ± 2^{a}	49 ± 1^{a}
	Wild		
Control	16 ± 2^{ab}	17 ± 2^{a}	51 ± 1^{a}
4	26 ± 4^{b}	28 ± 3^{b}	50 ± 1^{a}
8	19 ± 3^{ab}	20 ± 3^{ab}	48 ± 1^{a}
30	8 ± 2^{a}	8 ± 2^{a}	49 ± 1^{a}

characteristics of the wild, hatchery, and colonized coho salmon, although lymphocyte and leukocyte counts were marginally, but not significantly, decreased from the pre-stress values 4 h following handling.

Immune Function

There was a significant decrease in APC number in the anterior kidney of the wild coho salmon from pre-stress values in response to handling (Fig. 3). Similar decrease were seen in the hatchery and colonized groups but these were not statistically significant (Fig. 3). The pre-stress number of APC was significantly higher in the wild fish compared with the APC number of other two groups (Fig. 3).

Disease Challenges

Challenging the coho and chinook salmon of different rearing histories with *V. anguillarum* after 1 mo in the hatchery environment did not result in any significant differences in cumulative mortality among the groups representing the different rearing histories (Fig. 4). The cumulative mortality in chinook salmon, however, was about double that experienced by coho salmon.

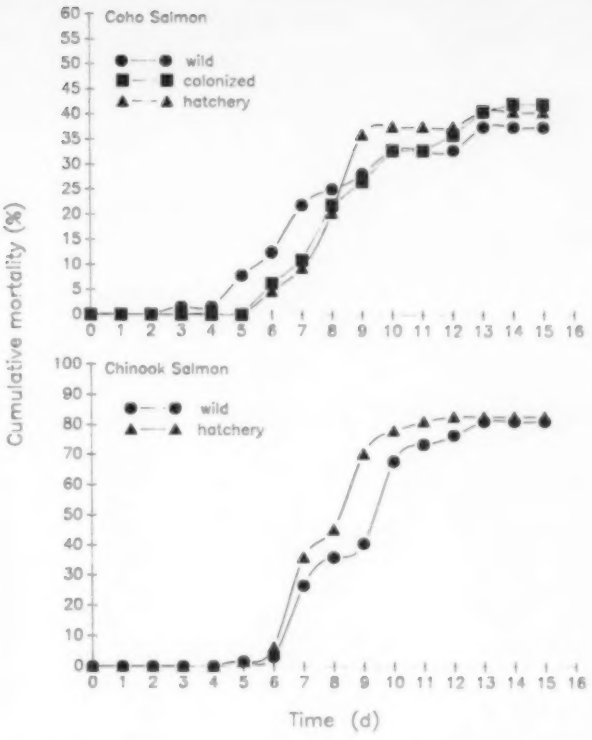

FIG. 4. Cumulative mortality over time of coho and chinook salmon following a challenge by bath immersion with *V. anguillarum*. Data represent means of pooled duplicate treatments (n = 64). All values are not significantly different.

After a further 6 mo in the hatchery environment, however, the cumulative mortality in coho salmon, as a result of the disease challenge, was significantly higher in the wild fish than in the hatchery-reared fish (Fig. 5). Mortality in the colonized group was intermediate to that in the hatchery and wild groups, and not significantly different from either (Fig. 5). An overall mortality of 10% occurred during August 1990 in the colonized coho salmon group, due to an outbreak of peduncle disease, and over the holding period the average growth rate in this group was 75% of that of the other two groups. During the 7-mo period, wild and hatchery coho salmon showed similar growth rates and negligible mortality.

Discussion

In these experiments, wild coho and chinook salmon juveniles had different physiological responses to a handling stress compared with their hatchery-reared counterparts. There was a heightened cortisol response in fish from the natural environment relative to that of fish reared in the hatchery, and this characteristic was retained after 7 mo in the hatchery environment.

There is evidence that the regulation of cortisol secretion is mediated by negative feedback of cortisol on the hypothalamic–

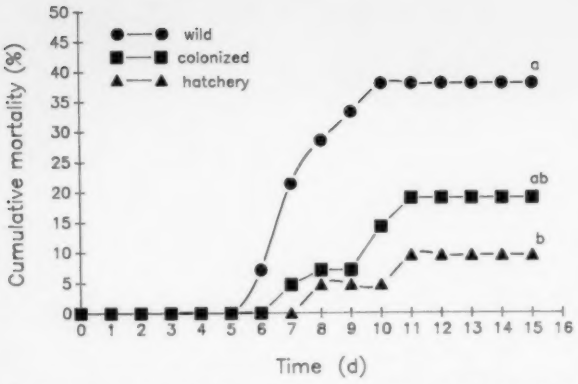

FIG. 5. Cumulative mortality over time following a challenge by bath immersion with *V. anguillarum* of coho salmon with different early rearing histories after 7 mo in a hatchery environment. Data represent means of pooled duplicate treatments ($n = 42$). Differing superscripts indicate significant differences ($P \leq 0.05$) among means.

pituitary–interrenal axis (Fryer and Peter 1977). It has also been shown that continued cortisol secretion from the interrenal cells inhibits further interrenal response (Donaldson 1981). Reduced interrenal response or acclimation to stress may be caused by a reduced secretion of adrenocorticotrophic hormone from the pituitary or by a reduction in cortisol-releasing factor from the hypothalamus, both of which would result in reduced levels of circulating cortisol (Barton et al. 1987). It is possible, therefore, that frequent episodes of stress that might be encountered in aquaculture, that may be acute (e.g., netting, grading, etc.) or chronic (e.g., crowding, poor water quality, etc.) in nature, may result in a down-regulation of the cortisol-based response to stress in hatchery-reared fish. This may have been the reason for the attenuated cortisol response in hatchery-reared coho and chinook salmon to the handling stress, relative to their counterparts which had a natural rearing history. A second possible explanation for the attenuated response in $[\text{cortisol}]_{pl}$ of the hatchery coho and chinook salmon is that there might have been greater clearance rates for $[\text{cortisol}]_{pl}$ in the hatchery fish compared with those in fish with a natural-rearing history. However, this explanation is unlikely, in light of the results of cortisol injection and metabolic clearance rate experiments under-taken by J.M Shrimpton in a parallel study with the coho salmon groups (J.M. Shrimpton, Department of Zoology, UBC, Vancouver, B.C. personal communication). He found that the rates of cortisol clearance in wild and hatchery fish were not significantly different, suggesting that the differences in $[\text{cortisol}]_{pl}$ between hatchery-reared fish and those that experienced a natural rearing history were related to differences in secretion and not clearance of cortisol.

These data also suggest that if the negative feedback effect of chronically elevated $[\text{cortisol}]_{pl}$ on subsequent cortisol secretion occurred in the hatchery-reared fish, it may have had an impact sometime during the development of the interrenal tissues rather than when those tissues were fully developed. Such a down-regulation in cortisol response did not occur in the wild and colonized coho salmon over the period of 7 mo in the hatchery; their cortisol responses to the handling stress were still higher than that of the hatchery fish (Fig. 2). Therefore, it is unlikely that the similarity between colonized and wild coho salmon in their cortisol response to the applied handling stress (Fig. 1) was due to a reversal of an attenuated cortisol response in the colonized group, which would have been a likely result of their early rearing in a hatchery environment. A more likely possibility may be that selective pressures in the natural environment favouring the survival of the wild phenotype determined the similarity in the responses of the colonized and wild coho salmon to the handling stress. In support of the existence of such selective pressures, there is evidence that shows that this management strategy of colonizing natural water bodies with hatchery fish does result in very high mortality rates between the time of release as fry and the smolt stage. The ocean survival (smolt to adult), however, of colonized fish at Quinsam Hatchery is significantly higher than that of hatchery-reared fish (D. Quinsam Hatchery, DFO, Campbell River, B.C., personal communication).

The transient increase in lymphocyte and leukocyte counts in both wild and hatchery chinook salmon 4 and 8 h after the handling stress, in this study, has been documented by others applying acute stressors to fish. Maule (1989) reported rapid and generally transient increases in leukocytes in the blood following acute stress in coho salmon. In contrast, no significant changes in lymphocyte or leukocyte counts were observed at 8 h in any of the coho salmon groups in this study. While this inconsistency cannot be explained by the present data, it is possible that there may be interspecies differences in the magnitude of catecholamine release in response to an applied stressor and that this may have influenced the white cell counts. There is evidence for the dependence of changes in the white blood cell numbers in circulation on the concentrations of both corticosteroids and catecholamines in humans (Fauci and Dale 1974; Yu et al. 1974). Increases in circulating lymphocytes are affected by contraction of hemopoietic tissues and subsequent emigration of lymphocytes into the blood (Fauci and Dale 1974; Yu et al. 1974). In rainbow trout (*Oncorhynchus mykiss*), increases in plasma epinephrine concentration caused contraction of the spleen and release of RBC into circulation (Perry and Kinkead 1989). This may also cause a concomitant increase in the number of circulating white blood cells, as reported in humans. Other investigators have reported consistent decreases in numbers of circulating leukocytes after stress, but these changes were evoked by subjecting fish to more severe handling or to chronic stress (McLeay 1975; Barton et al. 1987). Therefore, increases or decreases in circulating leukocyte numbers may be affected by plasma concentrations of both catecholamines and corticosteroids, and measurements of both should be included in future studies examining changes in leukocyte numbers in response to stress.

The results of the disease challenge experiments serve to demonstrate that complex biological functions, such as disease resistance, are not always easily explained by a few components of the immune system. Mortality from *V. anguillarum* has been shown to be stress mediated and a reliable indicator of adverse environmental conditions (Wedemeyer and McLeay 1981). Since the control values of $[\text{cortisol}]_{pl}$ in both coho and chinook salmon were similar (Fig. 1), it was not surprising to see similar trends among all groups in cumulative mortality to the disease challenge, although the chinook salmon were more susceptible to the disease, as evidenced by higher mortalities (Fig. 4). It is possible that the difference between the two species in the response of white cell counts to handling stress (i.e., no change in coho salmon, but significant transient increases in both hatchery and wild chinook salmon) might have

been related to the difference in their resistances to the pathogen.

In contrast with the results of the first disease challenge, the similar values of $[cortisol]_{pl}$ among the hatchery, wild, and colonized groups of coho salmon after 7 mo in the hatchery were associated with significantly different APC numbers (Fig. 3) and different patterns of cumulative mortality in the disease challenge among the three groups of coho salmon. These data show that while wild fish had significantly more APC numbers at rest than either hatchery or colonized coho salmon, they experienced the greatest mortality as a result of the disease challenge. This apparent inconsistency remains to be explained in future experiments, as greater APC numbers would have been expected to confer greater disease resistance. The association of the heightened cortisol response to the imposed handling stress in the wild coho salmon with the high APC numbers and lower disease resistance has some support in previous work. Although Fevolden et al. (1991) hypothesized that a high cortisol response in fish would have lower survival rates when challenged with disease, their study with Atlantic salmon and rainbow trout showed that certain immune responses were higher in the groups with the heightened cortisol response. Furthermore, Maule et al. (1989) found both immunosuppressive and immunostimulatory effects of stress in chinook salmon. The importance of the cellular immune response in defense against vibriosis may explain the relative decrease in disease resistance in the wild fish after prolonged holding when compared with their hatchery counterparts. Recent evidence has suggested that accessory cells (macrophages) may be the cellular targets of adrenergic hormones released immediately after a stressful disturbance (Flory and Bayne 1991). The events that impact immune function most significantly may be those closest in time to the onset of the stress. Chronically stressed fish have been shown to have levels of circulating cortisol that were lower than those of unstressed fish, while showing suppression of immune function (Pickering and Pottinger 1989). It is therefore possible that in this experiment, the pre-stress $[cortisol]_{pl}$ did not truly reflect the stress status of the animals, although these data also show that the scope for increasing $[cortisol]_{pl}$ was retained in those wild fish after 7 mo in a hatchery environment (Fig. 2). Decreased disease resistance may have been influenced by a stress-related modulation of macrophage activity, in addition to APC numbers, and possibly by other hormones such as catecholamines.

Genetically similar populations of coho and chinook salmon from different early rearing environments had distinctly different physiological responses when subjected to an acute handling stress. Wild fish are presumably the result of selection in the natural environment and might be considered as a model by which to measure the quality of hatchery smolts destined for release to supplement wild populations. Although the losses after release are high, the strategy of colonizing natural waters with hatchery-reared fry seems to result in a smolt that is more similar to the wild smolt than the hatchery-reared smolt. Future work to find ways of mitigating stress in the hatchery environment may produce hatchery fish with a higher capacity to survive the rigors of the natural environment. It is clear from these experiments that disease resistance cannot be explained simply by APC number and that a snapshot estimate of $[cortisol]_{pl}$ may not be a reliable indicator of the stress status of the fish. The modulation of the cell-mediated immune system would appear to be a promising area of study in elucidating the mechanisms whereby increased disease susceptibility may result from stress.

Acknowledgments

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Variation in Colour of *Pseudoterranova decipiens* (Nematoda; Anisakidae) Larvae Correlates with Haemoglobin Concentration in the Pseudocoelomic Fluid

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Dixon, B., W. Kimmins, and B. Pohajdak. 1993. Variation in colour of *Pseudoterranova decipiens* (Nematoda; Anisakidae) larvae correlates with haemoglobin concentration in the pseudocoelomic fluid. Can. J. Fish. Aquat. Sci. 50: 767–771.

Larval sealworm (*Pseudoterranova decipiens*) were collected from the fillets of Atlantic cod (*Gadhus morhua*) and the relationship between the colour of the nematodes, which varied from reddish brown to white, and the concentration of haemoglobin in their pseudocoelomic fluid was investigated. The colour of the haemoglobin, which makes up greater than 30% of the protein in the pseudocoelomic fluid, is derived from the heme group. As the heme copurifies with the protein and does not exist in the free form, colour variations in the nematode may, therefore, be due to differences in haemoglobin content. We investigate the possible regulation of levels of this protein and discuss the mechanisms by which this regulation would be achieved.

Des larves du ver du phoque (*Pseudoterranova decipiens*) ont été prélevées dans des filets de morue franche (*Gadhus morhua*) et la relation entre la couleur des nématodes, qui varie de brun rougeâtre à blanc, et la concentration d'hémoglobine dans le fluide pseudocoelomique a été étudiée. La couleur de l'hémoglobine, qui peut représenter plus de 30 % des protéines du fluide pseudocoelomique, provient du groupement hème. Comme le hème se copurifie avec la protéine et n'existe pas sous la forme libre, les variations de la couleur des nématodes peuvent donc s'expliquer par des écarts de la teneur en hémoglobine. Nous avons étudié la régulation des concentrations de cette protéine et traité des mécanismes pouvant la causer.

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Larvae of the parasitic nematode *Pseudoterranova decipiens* (commonly known as sealworm) infect several species of demersal fish in the North Atlantic, including Atlantic cod (*Gadus morhua*) (Templeman et al. 1957; Chandra and Khan 1988; Brattey et al. 1990; McClelland et al. 1990). The most important definitive hosts of the parasite appear to be grey seals (*Halichoerus grypus*) (Mansfield and Beck 1977; Marlouf 1986). Adult worms found anchored in the gastric submucosa (McClelland 1980) produce partly embryonated eggs which pass with the seals' feces. The eggs hatch into larvae that infect benthic macroinvertebrates. Secondary fish hosts ingest the macroinvertebrates and thus acquire the larvae. Larval sealworms mature into adults after entering the definitive host's stomach (McClelland 1990; Templeman 1990).

While investigating immunological methods for controlling sealworm infestation, we characterized the gene for one of the major antigens of the worm, a 40-kDa haemoglobin from the pseudocoelomic fluid (Dixon et al. 1991). In this report, we show a direct correlation between the content of this haemoglobin and the colour of individual worms which have been described variously as white, yellow, reddish-brown, or brown (Margolis 1977; Bowen 1990; Sinderman 1990; Appleton and Burt 1991). The function of haemoglobin in parasitic nematodes is still unclear. Most investigations of the function of nematode haemoglobin have involved adult *Ascaris suum* or *Ascaris lumbricoides*. *Ascaris* and *P. decipiens* belong to the same order of nematodes and their pseudocoelomic haemoglobins are similar in size and protein sequence (64% identity, with 83% conservative substitution) (Dixon et al. 1991, 1992). Thus, it is possible that haemoglobin in the pseudocoelomic fluid of *P. decipiens* is performing the same function as that found in *Ascaris*. Pseudocoelomic fluid haemoglobin, however, has such a high oxygen affinity that it is not likely to be deoxygenated in vivo and hence would not function in oxygen transport (Davenport 1949; Smith and Lee 1963). Some researchers have suggested that *Ascaris* haemoglobin serves as a hematin sink because these organisms are apparently unable to synthesize their own heme (Lee and Smith 1965), while others speculate that it supplies oxygen for the eggs as they pass through the anerobic lower gut of the seal (reviewed in Smith and Lee 1963). Evidently the amount of pseudocoelomic fluid haemoglobin can vary a great deal with no apparent adverse effect on the organism (Smith and Lee 1963). Recently, Burr et al. (1989) reported that nematode haemoglobins may also function in the photosensitivity of the ocelli of a free-living nematode. This suggests that the use of haemoglobin for functions other than oxygen transport by nematodes may not be unusual.

Materials and Methods

Source of Larval Sealworm

Larval nematodes were removed from fillets of Atlantic cod supplied by National Sea Products, Lunenburg Division, and

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stored at 4°C in Hanks buffered saline solution (HBSS). Worms of uniform size were separated according to colour into three groups: red, brown, and white. All worms were washed three times in HBSS.

Extraction of Pseudocoelomic Fluid

In the first experiment, 30 worms were sliced into segments less than 1 mm in length to release the pseudocoelomic fluid into 1 mL of sterile water. In the second experiment the body wall of 20 worms was sliced carefully with a scalpel along its length with the aid of a dissecting microscope. Pooled pseudocoelomic fluid was centrifuged at 14 000 rpm (16 000 x *g*) for 10 min to remove tissue and insoluble material.

Protein Content Determination

The concentration of protein in the pseudocoelomic fluid was assayed using a bicinchoninic acid protein assay kit (Sigma). One millilitre of pooled pseudocoelomic fluid from each group was scanned using a Beckman model DU-64 UV/visible spectrophotometer at wavelengths from 330 to 700 nm. The colour in haemoglobins is derived from the heme group (Lee and Smith 1965), which gives a characteristic spectrophotometric pattern.

Purification of Haemoglobin

The haemoglobin was purified from body fluid using the method of Darawshe et al. (1987). Pseudocoelomic fluid was bound onto DEAE-sephadex A50 in a column and eluted using 250 mM NaCl - 25 mM Tris-HCl. The eluate was then passed through a Sephadex G-200 column. The coloured fractions were pooled and concentrated in an Amicon Centriprep 10 concentrator.

Protein Composition and Western Blotting

The composition of the body fluid was determined by separating 10 μg of protein from each sample on a denaturing polyacrylamide gel (Laemmli 1970) and staining with Coumassie brilliant blue (Sigma). The relative amount of haemoglobin in the pseudocoelomic fluid was determined by Western blotting. Each well of the denaturing polyacrylamide gel was loaded with 10 μg of protein from samples of each colour group. The protein was transferred from the gel onto a nitrocellulose filter by electroblotting. This filter was screened using a polyclonal antiserum obtained by immunizing juvenile grey seals with purified *P. decipiens* pseudocoelomic fluid haemoglobin. Antibodies were detected using an alkaline phosphatase conjugated protein A (Sigma) followed by the substrates NBT and BCIP (BRL). Filters were blocked, washed, and probed by following the standard Western blot procedures (Towbin et al. 1979). The relative intensity of the haemoglobin band from each sample was obtained by scanning the developed filter with an Abaton 300-gs scanner (Everex Systems, Inc., Fremont, CA). The immunoreactive bands from the digitized image were analysed using Scan Analysis software (Biosoft, Ferguson, MO) on an Apple Macintosh IIsi computer (Apple Computer, Inc., Cupertino, CA).

RNA Preparation

Total cellular RNA was obtained from homogenization of 100 larval nematodes from each colour group by the lithium precipitation method of Palmiter (1974). The integrity and quantity of the RNA were analysed by electrophoretic fractionation on a denaturing 0.8% agarose gel at 90 V for 2 h.

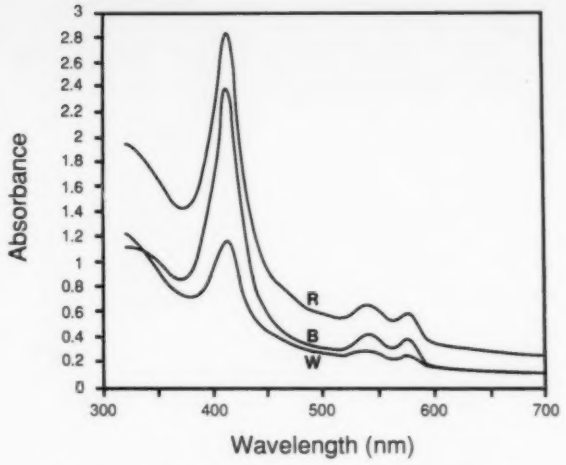

FIG. 1. Absorbance of *P. decipiens* body fluid from different colour groups at wavelengths from 300 to 700 nm. R, body fluid pooled from red worms; B, body fluid pooled from brown worms; W, body fluid pooled from white worms. The large peak at 400 nm and the secondary peaks at 540 and 580 nm are due to heme.

Northern Analysis

Approximately 20 μg of total RNA from worms of each colour group was denatured, loaded on a denaturing 0.8% agarose gel, and electrophoresed at 90 V for 2 h. RNA was transferred to a nylon membrane (Amersham). The blot was probed with [^{32}P]dCTP-labelled random primed (Feinberg and Vogelstein 1983) clone 10 cDNA insert (Dixon et al. 1991), isolated from pUC18 clones with *Eco*RI. The probe was hybridized in 50% formamide overnight and the blot was washed at 55°C in 0.2× SSPE, 0.1% SDS for 30 min. Blots were autoradiographed overnight at room temperature. Relative amounts of haemoglobin mRNA in each sample were determined by scanning the developed autoradiogram using the method outlined above for Western blots.

Results

Spectrophotometric analysis of pseudocoelomic fluid revealed that absorbance, and therefore heme concentration, was highest in the fluid from the red worms and lowest in fluid from white worms (Fig. 1). Similar results were obtained from the pooled pseudocoelomic fluid of the three groups of 20 longitudinally sliced worms.

Protein composition, as analysed by polyacrylamide gel electrophoresis and Coumassie brilliant blue staining (Fig. 2), did not differ in the pseudocoelomic fluid from the three sealworm colour groups, and in all cases, haemoglobin comprised more than 30% of the total protein. This is apparent in Fig. 2A; the predominant band is the 40-kDa haemoglobin molecule. Western analysis of each group of pooled pseudocoelomic fluid using polyclonal antiserum against the haemoglobin revealed that pseudocoelomic fluid from red worms contained the greatest concentration of haemoglobin, that from brown worms intermediate amounts, and that from white worms the lowest con-

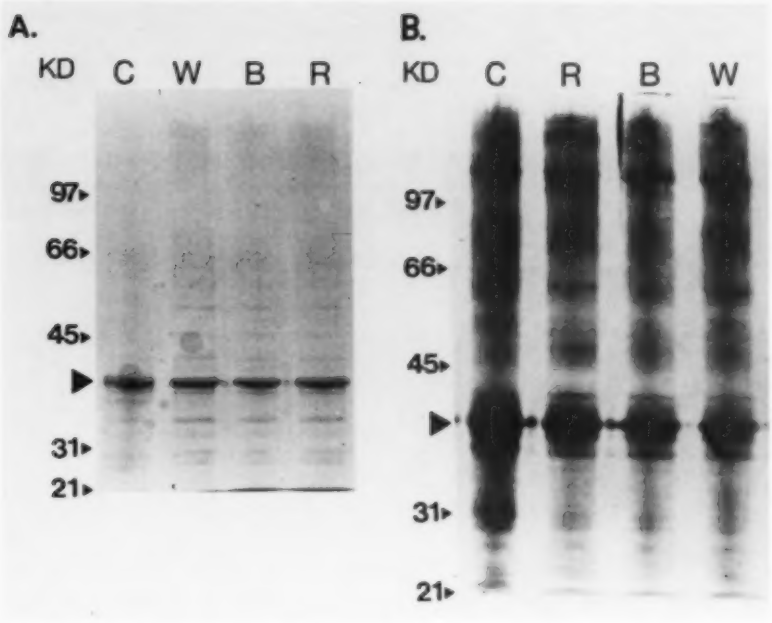

FIG. 2. Protein constituents of *P. decipiens* body fluid analysed by (A) polyacrylamide gel electrophoresis and (B) Western blot of body fluid probed with grey seal antihaemoglobin sera. Molecular weight in kilodaltons is indicated on the left. C, purified *P. decipiens* haemoglobin; R, body fluid pooled from red worms; B, body fluid pooled from brown worms; W, body fluid pooled from white worms. The large arrowhead indicates the *P. decipiens* haemoglobin.

centrations (Fig. 2B). These differences were quantified using densitometry. The ratio of haemoglobin concentration in the pseudocoelomic fluids of red, brown, and white worms was 1.0:0.77:0.56. Since equal amounts of protein were loaded in each well of the gel, this difference is in addition to differences in total protein concentration of the pseudocoelomic fluids from each sealworm colour group. The ratio of total protein concentrations in pseudocoelomic fluid was 1.0:0.84:0.66 (red:brown:white nematodes).

Probing of a Northern blot containing 20 μg of total RNA from each group revealed that there was no difference in haemoglobin mRNA content of the three colour groups of worms (Fig. 3). Quantification of the mRNA content by densitometry showed the ratio of band intensities to be 0.87:1.0:0.92 (red:brown:white nematodes).

Discussion

Variation of colour of larval sealworm from the flesh of Atlantic cod has caused some investigators to speculate that there are more than one species or variant of *P. decipiens* (Appleton and Burt 1991; Paggi et al. 1991). Multilocus electrophoresis has revealed the existence of three sibling species of *P. decipiens* in the North Atlantic, but of two siblings, *P. decipiens* B and C, found in Canadian waters, only sibling B has been identified from important commercial fisheries (Paggi et al. 1991). We report here a variation in body fluid protein content that does not indicate a difference in species, but may indicate the differential regulation of haemoglobin production. We tested the hypothesis that colour differences of *P. decipiens* larvae (red, brown, and white) were due to the con-

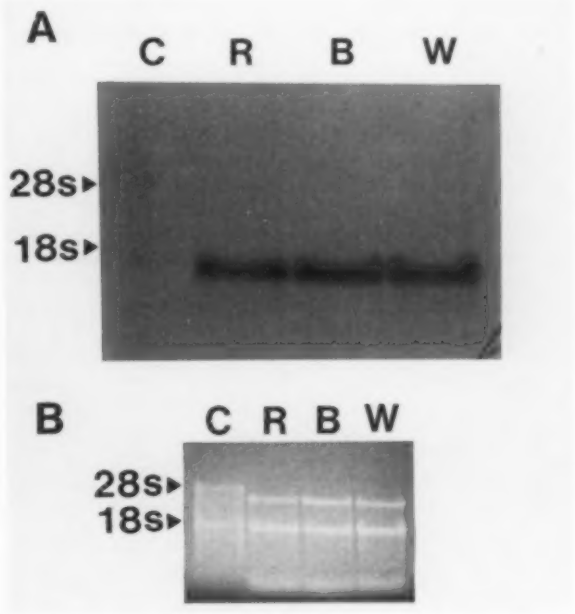

FIG. 3. (A) Northern blot indicating the relative levels of haemoglobin mRNA. C, human Jurkatt cell line RNA; R, RNA pooled from red worms; B, RNA pooled from brown worms; W, RNA pooled from white worms. (B) Agarose gel of 20 μg of total RNA from the three colour groups of worms. The arrowheads indicate the migration of the human 28s and 18s rRNA bands.

centration of haemoglobin, a prominent protein in the pseudocoelomic fluid of nematodes. The colour in haemoglobins comes from the heme group (Smith and Lee 1963) apparent in the spectrophotometric analysis of 1 mL of pooled pseudocoelomic fluid from 30 worms of each colour class (Fig. 1) as the large peaks at 400, 540, and 580 nm (Hamada et al. 1963; Wittenberg et al. 1965). Heme concentration was not due to free heme, since all the colour from the body fluid coelutes from the G-200 column with the haemoglobin molecule during purification.

We also noted that the pelleted body tissue contained very little colour (data not shown), and hence may differ from another ascaridoid nematode, *A. lumbricoides*, which has a second form of haemoglobin in the body wall. Alternatively, the concentration of body wall haemoglobin in *P. decipiens* may be significantly lower than the pseudocoelomic form. Nevertheless, our findings indicate a marked difference in the heme content of pseudocoelomic fluids of different-coloured worms.

Haemoglobin extracted from the nematodes could not include ingested host proteins, as all vertebrate haemoglobins are approximately 17 kDa in size (Dickerson and Geis 1983). *Pseudoterranova decipiens* haemoglobin is 40 kDa in size, as the gene encoding it has undergone an unusual head-to-tail duplication event (Dixon et al. 1992).

The protein composition of pseudocoelomic fluid protein herein appeared to be similar in all three colour groups of nematodes (Fig. 2A). Haemoglobin constitutes more than 30% of the *P. decipiens* pseudocoelomic fluid protein. This contrasts with the body fluid of *Ascaris* where an 11-kDa protein, which has proven allergenic in the definitive host, predominates (McGibbon et al. 1990). *Pseudoterranova decipiens* also has an 11-kDa allergen. Antibodies against the *Ascaris* allergen (ABA-1) show a strong reactivity to the *P. decipiens* 11-kDa protein on a Western blot (A. McGibbon, B. Dixon, T. Lee, and B. Pohajdak, unpublished data).

Coumassie staining of a gel containing equivalent amounts of protein from the pseudocoelomic fluid of each nematode colour group showed no apparent variation in the proportion of the haemoglobin band (Fig. 2A). Western blotting and screening with polyclonal sera to purified pseudocoelomic haemoglobin, however, revealed that the concentration of pseudocoelomic haemoglobin correlates with the variation in colour. The combination of the difference in proportion of haemoglobin in the pseudocoelomic fluid with the difference in total protein concentration between the three groups indicates that the brown worms have only 64.7% of the haemoglobin found in red worms, while white worms contain only 36.7% of the red worm level. Visually the difference in colour between the red and white worms appeared to be more than a 66% decrease in red colour, but the haemoglobin in the white worms may not contain heme and thus would not contribute to the colour. The correlation of haemoglobin content and variation in colour has been reported for *A. lumbricoides*, an ascaridoid nematode with a similar body fluid haemoglobin (Smith and Lee 1963).

Since haemoglobin is the major protein in the body fluid (Fig. 2), the increased protein content of red worms might reflect increased haemoglobin expression. To test this theory, we isolated total RNA from worms of each group and examined the haemoglobin mRNA content by Northern analysis and densitometry (Fig. 3). The difference in mRNA content, as measured by densitometry, is not significant enough to account for the large difference in protein content in the three groups of worms. Thus, difference in gene expression between the colour groups of worms is probably not due to differential regulation at the transcriptional level. Since haemoglobin mRNA from other organisms has been shown to have an extremely long half-life (Aviv et al. 1976; Lowenhaupt and Lingrel 1978), perhaps the difference in protein levels is due to regulation at the translational or posttranslational level.

Recently, we have reported the sequence of a genomic clone of *P. decipiens* haemoglobin (Dixon et al. 1992) and we are currently sequencing the upstream regulatory region of this gene to determine if its expression is oxygen or heme dependent. We are also testing whether various oxygen levels can alter in vivo synthesis of the haemoglobin mRNA and protein in the sealworm. Regulation of this gene by heme or heme precursors is suggested by the strong correlation of the decrease in colour derived from heme and the haemoglobin content. Heme and oxygen regulation of gene expression has been reported for the haemoglobin gene of *Escherichia coli* (Khosla and Bailey 1989) and for other genes (Zagorec et al. 1988; Minagawa et al. 1990; Belazzi et al. 1991). In *A. lumbricoides*, haemoglobin content increases following the addition of heme precursors (Smith and Lee 1963), as, evidently, this nematode cannot synthesize heme de novo due to an inability to synthesize porphyrin ring heme precursors (Lee and Smith (1965).

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Estimation of the Functional Form of Selectivity by Baited Hooks: A Graphical Approach

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Kenchington, T.J. 1993. Estimation of the functional form of selectivity by baited hooks: a graphical approach. Can. J. Fish. Aquat. Sci. 50: 772–780.

A recently proposed graphical technique for exploring the selectivity of hooked fishing gears, involving the plotting of catch ratios against fish length, is further developed and illustrated. This technique can provide first estimates of the functional form of selectivity curves and sometimes estimates of some parameter values as well. It is therefore a useful preliminary to nonlinear estimation of hook selectivities.

L'auteur pousse plus avant l'élaboration et l'illustration d'une technique graphique, qui fait appel au traçage des rapports de prises en fonction de la longueur des poissons, récemment proposée pour l'étude de la sélectivité des engins de pêche à hameçons. Cette technique permet d'obtenir une première estimation de l'allure fonctionnelle des courbes de sélectivité et, parfois, d'estimer la valeur de certains paramètres. Elle s'avère donc utile comme estimation non linéaire préliminaire de la sélectivité des hameçons.

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The size-selective properties of fishing gears have long been studied. Most of this work has concentrated on trawl nets, where the escapement of small fish through the codend meshes has been of primary concern. This narrow focus has allowed the use of simple conceptual and mathematical models of the selection process. Gillnet selection has also been studied in depth, although generally with a wider focus, encompassing all those processes by which the size distribution of the catch differs from that of the population. Viewed in that way, selection requires a more elaborate mathematical representation and hence increased difficulties of estimation. It is nevertheless possible to build conceptual models of gillnet capture processes and thus to propose appropriate functional forms for the mathematical representation of selectivity (e.g., Hamley 1975; Pope et al. 1975).[1]

In contrast, selection by baited hooks has been largely ignored, with even the processes by which such gear selectively catches different sizes of fish being poorly known. There is presumably some link between hook size and fish size, but the size and type of bait, the arrangement of the gear, and even the presence of other fish seem to exert important influences on selectivity in at least some situations (Løkkeborg and Bjordal 1992). Given these uncertainties about processes, the functional form of selectivity by baited hooks remains unclear; some authors have suggested that it is asymptotic (e.g., McCracken 1963; Saetersdal 1963), while others have found it to be dome-shaped, possibly following the normal or some other curve (e.g., Kanda et al. 1978; Koike and Kanda 1978). Furthermore, whatever its form, selection by hooks often seems rather weak, some studies having failed to detect any differential selective effect between hooks of different sizes (e.g., Pope et al. 1975; Ralston 1982; Bertrand 1988). Such weak effects are inevitably difficult to describe quantitatively.

It is at least certain that the simple concepts and methods used for the estimation of trawl mesh selectivities are inappropriate for studies of baited hooks. It has therefore become normal to apply instead the selectivity estimation techniques developed for gill nets, despite the lack of evidence for any similarity of shape in the selectivity curves of these two types of gear. Moreover, none of the conventional gillnet methods is fully satisfactory even for the gear for which it was designed (Kirkwood and Walker 1986). Given modern computational facilities, Kirkwood and Walker (1986) have suggested discarding these methods in favour of simply fitting some chosen selectivity function to field data using nonlinear maximum likelihood estimation. Their approach avoids most of the problems inherent in the conventional estimation of gillnet selectivities but is of even greater utility in studies of hooked gear. This is because it permits the use of a wide range of functional forms for selectivity, with as many or as few constraints on parameter values as are appropriate, and especially because it offers an objective basis for determining which forms and constraints provide the best and most parsimonious fit to the data. Thus, it is possible to apply Kirkwood and Walker's (1986) approach in the absence of knowledge of the selective processes involved in fish capture by baited hooks. The resulting selectivities will be no more than descriptions of the data, as distinct from mechanistic models, but that is inevitable given the lack of knowledge of the processes involved.

The principal disadvantage of nonlinear estimation for this purpose is that it is slow, with each fit of a typical model taking several hours for even a powerful microcomputer. It also demands considerable interaction between computer and investigator. Any step that can provide rapid, preliminary indications of the appropriate functional form for selectivity or of initial estimates of parameter values would greatly speed and simplify

[1]Following Hamley (1975), I use the term "selectivity" here to mean the quantitative expression of the result of the size-specific differential selection of fish by a fishing gear.

the final computations. Such a step might also be of use in settings where powerful computing facilities are unavailable, potentially giving first, crude indications of selection patterns.

Ralston (1990) recently proposed a graphical method for determining the general shape of selectivity curves for baited hooks. In his method, the ratios of the catches-at-length taken by the larger of two baited hooks to those taken by the smaller are plotted against fish length. He provided theoretical plots of the catch ratios expected from logistic (asymptotic) selection, where the selectivities of the various gear sizes were assumed to differ only in the fish size at 50% retention, and from normal (domed) selection, where the selectivity curves were assumed to differ only in their optimal fish lengths. Unfortunately, Ralston's (1990) own field data, derived from hook-and-line fishing for four species of lutjanid snapper, did not closely accord with either of these model forms, leaving an impression that his graphical approach was not helpful.

In this paper, I extend Ralston's treatment to include a wider variety of selectivity functions and gear-related variations in more parameters of those functions. I then compare these theoretical plots with previously published data and suggest that this graphical approach can indeed be a useful precursor to nonlinear estimation of selectivity. I do not attempt such estimation here.

Plots analogous to those presented by Ralston (1990) have been used elsewhere but only as a comparison between gears with different selectivity characteristics (e.g., trawl nets and hooks: Hovgård and Riget 1992) or with an assumption that one gear was nonselective across the range of fish sizes of concern (e.g., Templeman 1963). The plotting of catch ratios from two selective gears as a means of estimating the common functional form of their selectivities was a new departure by Ralston (1990). Thus, I propose here the term "Ralston Plot" for graphs of catch ratio against fish size used in this way.

Theoretical Plots

Selectivity can be defined by the equation

$$C_{ij} = S_{ij} \cdot q \cdot N_j$$

where C_{ij} is the catch per unit effort of fish of length j by gear of size i, N_j is the number of fish of length j available to a unit of effort of the gear, s_{ij} is the selectivity of gear size i for fish size j, and q is a scaling factor chosen such that some predetermined s_{ij} (usually $s_{1,\text{opt}}$, the selectivity of the smallest gear studied for fish of that gear's optimal length) is set at unity. If a selectivity experiment is conducted such that the same N_j vector is available to each gear size studied and that the gear sizes differ only in the intended characteristic indexed by i (e.g., hook gap or bait size), then $C_{i+1,j}/C_{ij}$ is an estimate of $s_{i+1,j}/S_{ij}$. Thus, the ratios of catches-at-length by two gear sizes can be used to estimate the ratios of the applicable selectivities while, if theoretical selectivity models are generated, dummy Ralston Plots can be produced by plotting the ratios of the selectivities for each length.

Various functional forms have been suggested in the past for modelling the s_{ij} matrices, of which the normal, skew normal, and gamma have been most prominent for gillnet selection; the logistic has been almost universal for trawl mesh selection, with the Gompertz being preferred if the selectivities are asymmetric across the fish size at 50% retention. Theoretical Ralston Plots derived from each of these selectivity functions are presented in this section.

Absence of Selection

If the gear being studied were genuinely nonselective across the range of fish lengths caught (i.e., s_{xj} equal for all j and each x), then the ratios of catches-at-length would be equal for all fish lengths, resulting in a Ralston Plot that took the form of a horizontal line. If the various sizes of gear were also equally efficient, that line would lie at a value of unity, with displacements up or down the catch ratio axis indicating an overall efficiency difference between the gear sizes. However, exactly the same form of Ralston Plot would be produced if the gear were highly selective, regardless of the shape of the selectivity curve, provided that the various sizes of gear had identical selection properties (i.e., s_{iy} equal for all i and each y) or if they differed only in their overall efficiency. Thus, a horizontal line in a Ralston Plot indicates not an absence of selection but an absence of information in the data set. A first step in applying Ralston Plots should therefore be to determine whether the plot departs significantly from a horizontal line.

Logistic Selection

In this paper, "logistic selection" is taken to be asymptotic selection that follows the cumulative function of the logistic distribution:

$$s_{ij} = 1/\{1 + \exp[-\alpha(l_j - \beta)]\}$$

where l_j is fish length, β is the length at 50% selection, and α is a parameter that controls the selection range.

A comparison of logistic selection curves that differ only in fish length at 50% retention (Fig. 1Ai) leads to Ralston Plots that are indistinguishable from the parent curve representing the numerators in the catch ratios, unless the selection ranges overlap broadly (Fig. 1Aii; equivalent to fig. 2 of Ralston 1990). While different values of α produce different shapes of selection curve (Fig. 1Bi), the close similarity between the Ralston Plot and its parent curve is unaffected. If, however, a Ralston Plot is prepared from two logistic curves with different values of α, it can show an indefinite increase in catch ratio toward very small fish lengths (Fig. 1Bii). This is an artifact of the model which is most unlikely to occur in reality. It is, however, conceivable that extremely small fish might be equally vulnerable to both small and large gears, causing a Ralston Plot of field data to rise to unity at low fish lengths.

Gompertz Selection

This is an alternative form of asymptotic selection that follows the cumulative form of the Gompertz function:

$$s_{ij} = \exp[-\alpha \cdot \exp(-\beta l_j)]$$

where α and β are parameters with similar functions to, but are not directly equivalent to, the homonymous parameters of the logistic function.

Gompertz selection curves lead to Ralston Plots that differ from those generated by the logistic function only in having a slightly gentler curve near 90% than near 10% selection (Fig. 1C). As with logistic selection, a comparison of two gears with different selection ranges can lead to increasing catch ratios at very small fish lengths (not illustrated).

It is unlikely that Gompertz selection could be distinguished from logistic selection by a Ralston Plot of field data, but this pair of functional forms should be collectively recognizable in such plots, provided that data on fish lengths corresponding to

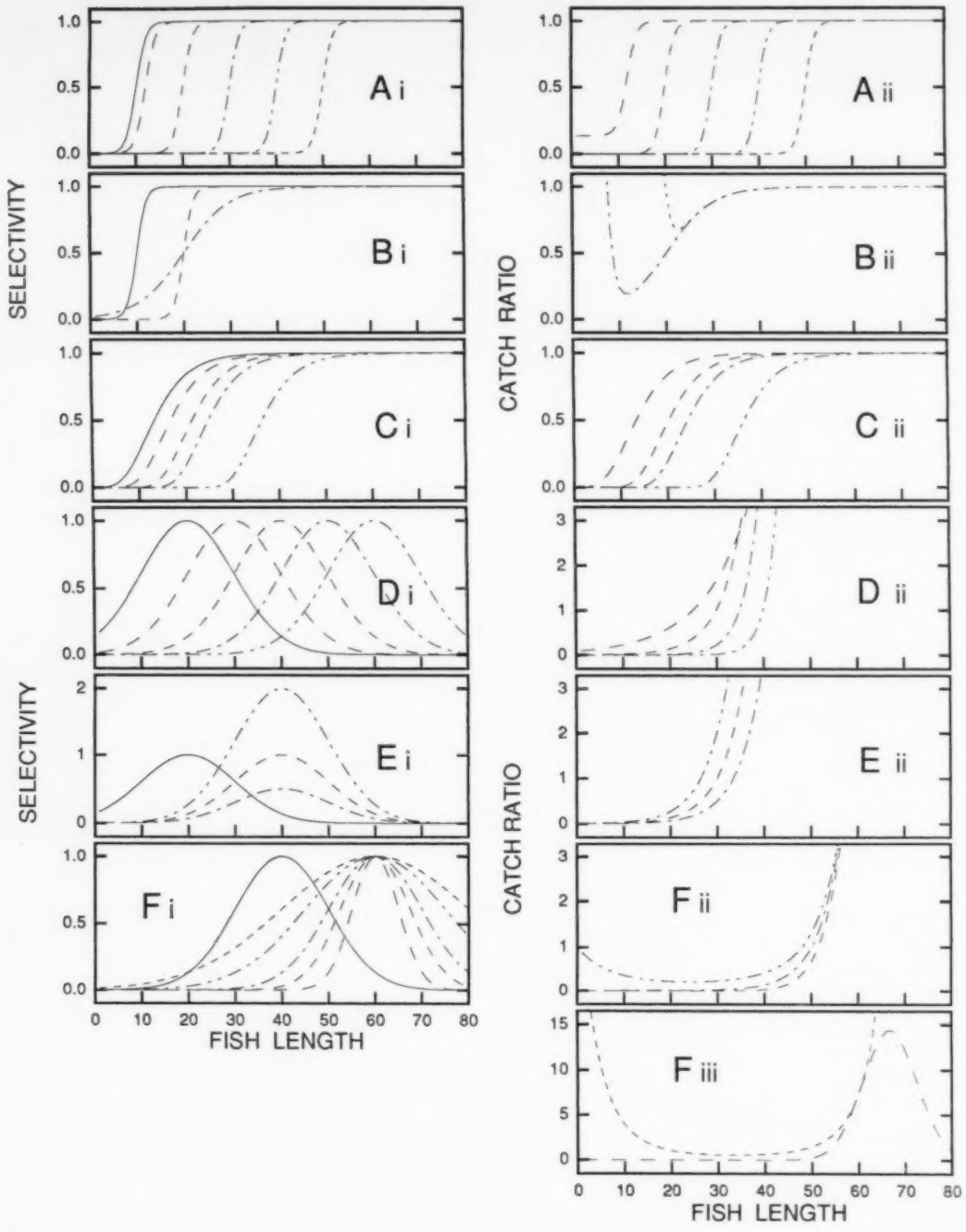
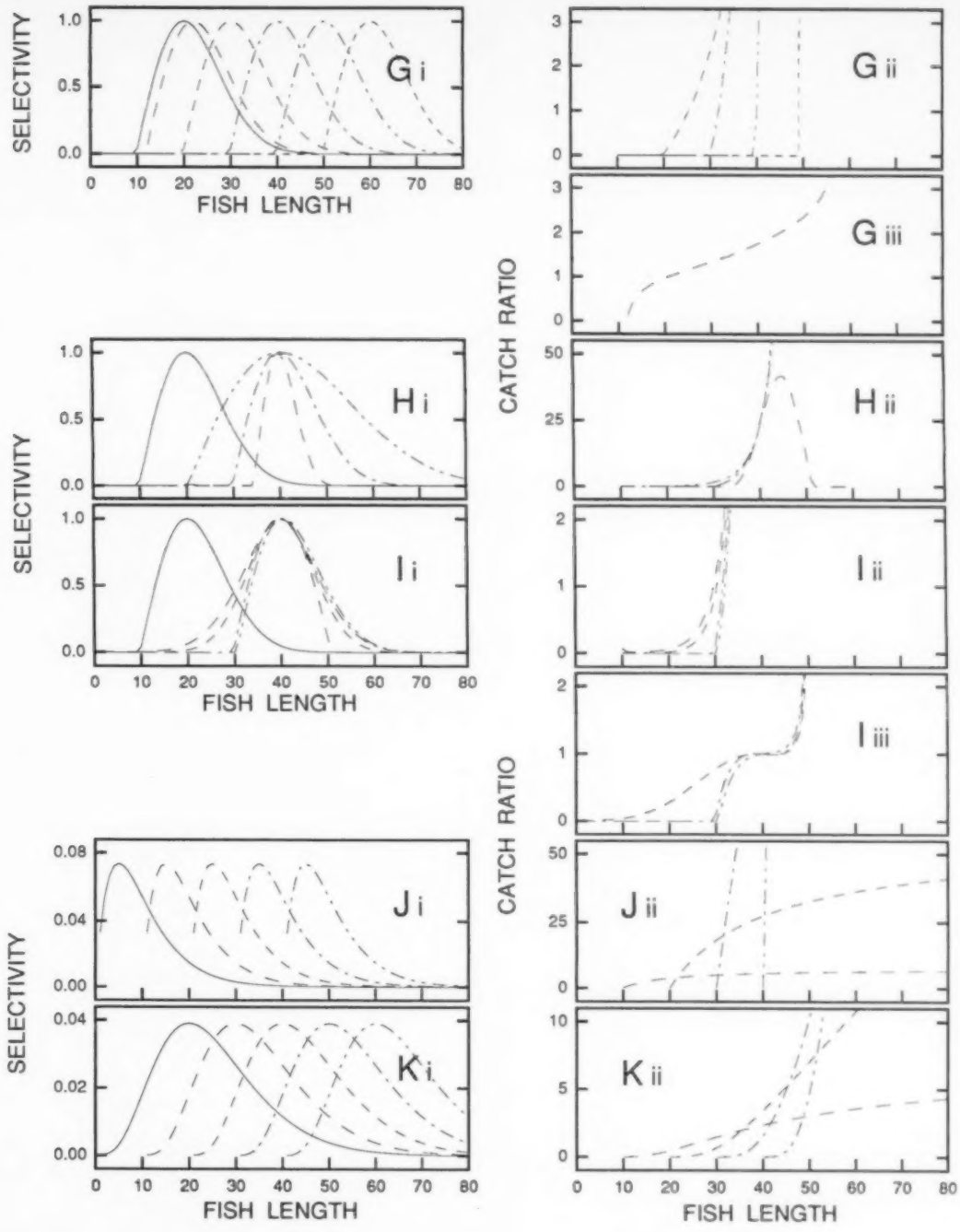


FIG. 1. Families of theoretical selectivity curves with various functional forms (left-hand columns) and the corresponding Ralston Plots (right-hand columns). Abscissae are in arbitrary units of fish length. Except in plots Bii and Iiii, as noted below, each curve on a Ralston Plot represents the ratio of the function graphed in the corresponding selection curve family with the same line type to that graphed there with a solid line. (A) Logistic selection with $\alpha = 1$ and $\beta = 10$, 12, 20, 30, 40, and 50. (B) Logistic selection with $\alpha = 1$ and $\beta = 10$ and 20 or with $\alpha = 0.2$ and $\beta = 20$. (In Bii, the pecked line represents the ratio of the two selection curves in Bi that differ only in their values of α.) (C) Gompertz selection with $\alpha = 0.2$ and $\beta = 10$, 20, 50, 100, and 1000. (D) Normal selection, scaled to $s_{i,\mathrm{opt}} = 1$ for all i, with $\beta = 10$ and $L_i = 20$, 30, 40, 50, and 60. (E) Normal selection with $\beta = 10$ and $L_i = 20$ or 40 and $s_{i,\mathrm{opt}}$ scaled to 0.5, 1, or 2. (F) Normal selection, scaled to $s_{i,\mathrm{opt}} = 1$ for all i, with $L_i = 40$ and $\beta = 10$ or with $L_i = 60$ and $\beta = 5$, 7.5, 10, 15, and 20. (*Fig. 1 continued next page*)



(G) Skew normal selection, scaled to $s_{i,opt} = 1$ and with $k_i = -0.5$ for all i, with $\beta = 10$ and optimum lengths of 20, 22, 30, 40, 50, and 60. (H) Skew normal selection, scaled to $s_{i,opt} = 1$ and with $k_i = -0.5$ for all i, with optimum length of 20 and $\beta = 10$ or with optimum length of 40 and $\beta = 5$, 10, and 20. (I) Skew normal selection, scaled to $s_{i,opt} = 1$ for all i, with optimum length of 20, $k_i = -0.5$, and $\beta = 10$ or with optimum length of 40, $\beta = 10$, and $k_i = +1$, -1, and -2 or with optimum length of 40, $\beta = 7$, and $k_i = 0$. (In Iiii, the Ralston Plots represent ratios in which the divisor is represented in Ii by the line with $k_i = +1$.) (J) Gamma selection with $\alpha = 2$, $\beta = 5$, and $\gamma = 0$, 10, 20, 30, and 40. (K) Gamma selection with $\alpha = 5$, $\beta = 5$, and $\gamma = 0$, 10, 20, 30, and 40. (L) Gamma selection with $\alpha = 5$, $\beta = 5$, and $\gamma = 10$ or with $\alpha = 3$, 5, and 7, $\beta = 5$, and $\gamma = 30$; all curves scaled to $s_{i,opt} = 1$. (M) Gamma selection with $\alpha = 5$, $\beta = 5$, and $\gamma = 10$ or with $\alpha = 5$, $\beta = 2.5$, and 10, and $\gamma = 30$; all curves scaled to $s_{i,opt} = 1$. Note different scales. (*Fig. 1 concluded next page*)

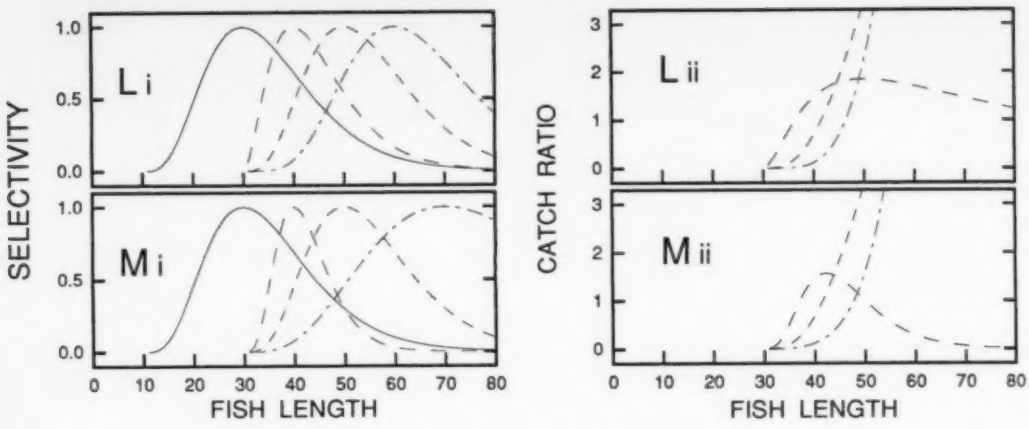


FIG. 1. (*Concluded*)

the asymptote of the larger gear's selectivity are included. These plots might also serve to indicate initial estimates of α and β for the larger gear.

Normal Selection

"Normal selection" is taken here to be nonmonotonic selection that follows a scaled probability density function of the normal distribution:

$$s_{ij} = \{h_i/(\beta\sqrt{2\pi})\}\cdot\exp\{-(l_j - L_i)^2/2\beta^2\}$$

where h_i is a scaling parameter that controls the value of s_{ij} at the optimum fish length for gear size i ($s_{i.\mathrm{opt}}$), L_i is the optimum length for gear size i, and β^2 is a parameter that controls the selection range. (β^2 fulfills the same rôle in the function as does the variance, σ^2, in statistical applications and has sometimes been referred to by that name in selectivity studies. As applied here, however, it has nothing to do with variation, and to avoid confusion with the variance of the error term associated with field data, it is denoted by a different symbol. It has no direct equivalence to the β parameters of the logistic or Gompertz functions.)

If the selectivity curves of the different gear sizes differ only in their optimum lengths (Fig. 1Di), they lead to Ralston Plots similar to those shown in Fig. 1Dii (equivalent to fig. 3 of Ralston 1990). At low fish lengths, the catch ratios are small but positive. They increase through unity at a length midway between the optimal lengths of the two gear sizes being compared and then continue to increase without limit. The greater the separation between the optimal lengths (relative to the common value of β), the sharper will be the curvature in these plots.

It is, however, possible that the selectivity at optimum fish size ($s_{i.\mathrm{opt}}$) differs with gear size (Fig. 1Ei), representing an overall difference in the efficiency of the two gear sizes. Such a change has no effect on the corresponding Ralston Plot except that all of the catch ratios are scaled up or down by the ratio of $s_{2.\mathrm{opt}}$ to $s_{1.\mathrm{opt}}$ (Fig. 1Eii). The curve will, therefore, pass through a value equal to this ratio at a fish length midway between the optimal lengths of the two gear sizes. Catch ratios scaled in this way produce Ralston Plots that are of different curvature from any in Fig. 1D, but it is unlikely that such a difference could be reliably detected in field data.

Cortez-Zaragoza et al. (1989) have suggested that large hooks are less strongly selective than smaller ones, implying that the corresponding normal curves should have different values of the selection range parameter (Fig. 1F). Over a considerable range of values, variations in β have negligible effects on the corresponding Ralston Plot. Extreme differences can, however, give rise to anomalous behaviour in which large hooks with a high selection range appear to catch more small fish than small hooks do or vice versa. The corresponding catch ratios either increase without limit as fish size decreases or else they form a dome, returning to very low levels as fish size increases (Fig. 1Fiii). As with the analogous phenomenon in logistic selection, these behaviours are artifacts of the mathematical model and are unlikely to arise in reality. It is conceivable, however, that the catch ratios might tend towards unity at fish sizes far from the optimal size for either test gear. If that were seen in a Ralston Plot of field data, normal curves with very different values of β might be an appropriate model form over the range of fish sizes of primary interest.

In summary, normal selection leads to a particular shape of Ralston Plot that is distinctively different from that generated by logistic or Gompertz selection. It might also be possible to detect extreme differences in selection range between the tested gear sizes with such plots, but otherwise they only seem able to indicate the appropriate family of functions.

Skew Normal Selection

This form of nonmonotonic selection, suggested for gillnet selectivity by Regier and Robson (1966), follows a scaled probability density function of the normal distribution modified by a skew term comprising the first two terms of the Gram–Charlier series:

$$s_{ij} = \{h_i/(\beta\sqrt{2\pi})\}\cdot\exp\{-(l_j - L_i)^2/2\beta^2\}\cdot\{1 - [k_i\beta^{1.5}/2]\cdot[(l_j - L_i)/\beta - (l_j - L_i)^3/3\beta^3]\}$$

where k_i is the skewness coefficient for gear size i. The skew term causes the optimum length to deviate from L_i and the value of $s_{i.\mathrm{opt}}$ to be controlled by k_i and β as well as h_i. For some fish lengths, this function yields negative values that have no meaning as selectivities. All such values have been arbitrarily replaced by zero for present purposes.

Skew normal selection (Fig. 1G, 1H) usually yields Ralston Plots very similar to those derived from normal selection, whether the selection ranges are constant or not. The scaling effect of differences in $s_{i,opt}$ operates exactly as with normal selection (not illustrated). The curvatures of plots derived from skew normal selectivities are rather sharper than those from normal curves but this difference is unlikely to be detectable with field data.

If the optimal lengths of the two gear sizes are closely similar ($\leq 0.5\ \beta$ apart), however, the corresponding Ralston Plot will show a reverse curve at very small fish lengths (Fig. 1Giii). The point of inflection of such a curve will lie at a fish length midway between the optimal lengths of the two gear sizes and at a catch ratio equal to $s_{2,opt}$:$s_{1,opt}$. This phenomenon does not occur with normal curves and, if detected in field data, would be a clear indication that the skew normal is a more appropriate functional form. It should be noted, however, that the curve of such a Ralston Plot is asymmetric, the inflexion point occurring near the reverse curve at low fish length. Field data might easily be misinterpreted as showing an inflexion at a higher catch ratio and a larger fish size.

Changes in the skew parameter itself have little effect on Ralston Plots if the two gear sizes have widely separated optimal lengths (Fig. 1I). (Because of the effects of the skew term on selection range, the zero-skew curve in Fig. 1Ii has been given an arbitrarily lower value of β to make its shape comparable with those of the other curves. No such adjustment for the $k_i = -2$ curve appeared necessary). The complex effects observed at extreme fish lengths with normal curves of different β are rarely seen in this rather analogous situation because the values of the skew normal function for such extreme lengths have mostly been replaced with zero. In another form of artifact, one curve in Fig. 1Iii should return precipitously to zero with increasing fish length, much as does the analogous curve in Fig. 1Hii, but this descending limb has been deleted from the figure for clarity.

If the gear sizes differ in their values of the k_i parameter but not in optimal length, however, the Ralston Plot will appear as a tight, inflected curve with its point of inflexion at the joint optimal length and at a catch ratio equal to $s_{2,opt}$:$s_{1,opt}$, the sharpness of the curvature on either side of the inflexion depending on the difference between k_1 and k_2 (Fig. 1Iiii). With real data, it might be difficult to determine whether a Ralston Plot indicated close-set optimal lengths, a narrow selection range, and constant skew (cf. Fig. 1Giii) or near-identical optimal lengths, a wider selection range, and varied skew (cf. Fig. 1Iiii). Both possibilities would have to be tested when fitting the models.

In summary, skew normal selection will not generally be separable from normal selection by means of Ralston Plots. Those plots could be diagnostic, however, if the difference between the optimal lengths is small, relative to the selection range, or if it is very small and the selectivities of the two gear sizes are differently skewed. Because hooks seem often to have rather wide selection ranges, these conditions may be fulfilled in many cases.

Gamma Selection

"Gamma selection" is nonmonotonic selection following the probability density function of the gamma distribution:

$$s_{ij} = (l_j - \gamma)^{\alpha-1} \cdot \exp\left[-(l_j - \gamma)/\beta\right] / \{\beta^{\alpha} \cdot (\alpha - 1)!\}$$

where γ is a parameter analogous to L_i in the skew normal function, α controls the degree of skew (the gamma distribution tends towards the normal as α tends towards infinity) but also influences optimum length and $s_{i,opt}$, and β influences optimum length, selection range, and $s_{i,opt}$. Neither α nor β has any relation to the homonymous parameters in the other functions examined here. Because of the complex relationships between the three parameters, which prevent them from being interpreted directly, there is no virtue in adding a scaling parameter to set $s_{1,opt}$ at unity; if that requirement were set as a constraint on nonlinear estimation, appropriate values of α, β, and γ would be found. This function does not yield selectivity values for fish lengths less than γ.

Gamma selectivity curves (Fig. 1Ji) differ little in overall shape from skew normal curves. For low values of α, however, and when selectivity functions that differ only in γ are compared, they lead to quite different Ralston Plots that take the form of asymptotic curves, the values of the asymptotes of which increase with the difference in γ between the gear sizes (Fig. 1Jii). At higher α, the Ralston Plots acquire an inflexion at small fish sizes (Fig. 1K) and, as α approaches infinity, they lose their asymptotes and take the form of Ralston Plots derived from normal curves. As the convexity in some of these plots occurs at very high values of catch ratio (e.g., Fig. 1Jii), however, it might not be apparent in typical field data.

If gamma curves that differ in only α or β are compared, they differ markedly in $s_{i,opt}$ values (sometimes by several orders of magnitude). To provide more meaningful comparisons, Fig. 1L and 1M show selectivity curves with one of these parameters altered while the other is held constant and the calculated values then scaled to produce a common $s_{i,opt}$. Either alteration usually leads to a Ralston Plot reminiscent of those generated by normal selection, while extreme differences in parameter values produce unrealistic, domed plots. If all three parameters of the gamma function were varied simultaneously while maintaining some fixed relationships among optimum length, selection range, and $s_{i,opt}$, as would occur when fitting field data, the resulting Ralston Plots could take a wide variety of forms. Indeed, because the gamma distribution approaches the normal as α increases and resembles the skew normal if given other parameter combinations, it is perhaps better seen as a general function that could fit data that show any of a wide range of Ralston Plot forms. Certain such plots will clearly indicate, however, that the gamma is an appropriate function when the others reviewed here are not.

Some Applications to Data

There are very few published data sets derived from comparative fishing of different sizes of baited hooks with which to test the utility of Ralston Plots, and none at all that are fully adequate for the estimation of selectivities. Nevertheless, I present plots here of a selection of those data sets that are available. Because most of the diagnostic behaviour of the theoretical plots occurs at the smallest and largest fish lengths, I plot here the catch ratios for all lengths for which data are available, rather than deleting the data for those at which few fish were caught.

The theoretical Ralston Plots presented above naturally follow smooth curves. Field data, on the other hand, will necessarily contain some experimental error, which will complicate the interpretation of the plots. This is a particular problem because of the use of ratios. Even if the error associated with the catches-at-length were normally distributed, the catch ratios would have associated error terms with Cauchy distributions and thus would tend to show extreme variation. In practice, the

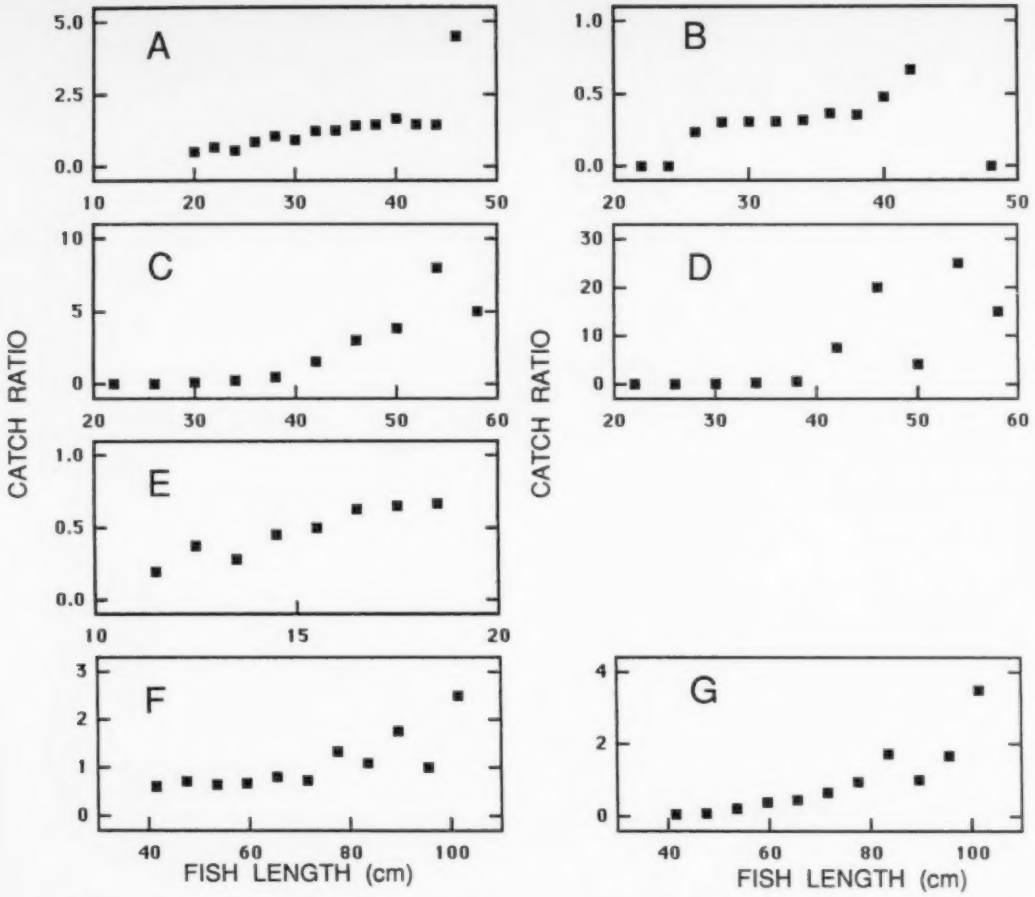


FIG. 2. Examples of Ralston Plots prepared from various published data sets on selection by baited hooks. (A) Fishing for *Pristipomoides zonatus* with No. 20 and No. 28 Izuo circle hooks (Ralston 1990). (B) Fishing for *Pristipomoides auricilla* with No. 20 and No. 28 Izuo circle hooks (Ralston 1990). (C) Handlining for *Thunnus albacares* with 13- and 14-mm-gap hooks (Cortez-Zaragoza et al. 1989). (D) Handlining for *Thunnus albacares* with 13- and 16-mm-gap hooks (Cortez-Zaragoza et al. 1989). (E) Longlining for *Acanthogobius flavimanus* with No. 13 and No. 5 Ryusen–Sodegata hooks (Takeuchi and Koike 1969). (F) Longlining for *Gadus morhua* with No. 14 and No. 17 hooks (McCracken 1963; Koike et al. 1968). (G) Longlining for *Gadus morhua* with No. 11 and No. 17 hooks (McCracken 1963; Koike et al. 1968). Note different scales.

catches-at-size are more likely to be Poisson-distributed, and at the extreme fish lengths that are of particular interest, they are likely to be small, giving them markedly discrete distributions that do not closely approximate to normality. The ratios of catches with such distributions will be highly unstable. (Consider, for example, the ratios obtained when one size of hook catches a single fish of a certain length and the other chances to catch none, one, or two of that length.) Thus, Ralston Plots may be useful tools when used as precursors to more formal analysis, but they must always be interpreted with great care.

Some authors who have used catch ratios for other purposes have preferred to plot the logarithms of these ratios against fish size (e.g., Hovgård and Riget 1992). In the context of Ralston Plots, this transformation could serve to constrain the apparent variability of the ratios for larger fish sizes and thus make the plots easier to interpret. Unfortunately, if the curves of Fig. 1 are replotted on log-linear axes, they lose most of their diagnostic differences and hence their practical utility. Furthermore, to avoid division by zero, any use of catch ratios requires the discarding of data on large fish taken on the larger gear if the smaller gear caught no fish of the same size. Plotting logarithms of catch ratios would require the further discarding of some data on small fish taken on the smaller gear because the logarithm of zero is undefined. Thus, Ralston Plots cannot usefully be prepared on logarithmic scales.

Ralston (1990) applied his graphical approach to data on hook-and-line fishing for four species of lutjanids, using No. 20 and No. 28 Izuo circle hooks. He concluded that the resulting plots were not consistent with normal selection if the hook sizes differed only in their optimum fish lengths, nor with logistic selection if they differed only in the fish length at 50% selection. Figure 2A reproduces his plot of the data for *Pristipomoides zonatus*, with the addition of some data points for extreme fish lengths that he excluded. As noted above, the first step in the interpretation of this plot should be to test whether it deviates from a horizontal line and thus contains useful information. There is no universal test for information content appropriate to all Ralston Plots, but a significant value of the

nonparametric correlation coefficient, Spearman's ρ, would be sufficient to demonstrate a relationship between catch ratio and fish size. This coefficient is preferable to its parametric equivalent, r, both because of the nonnormal distribution of the catch ratios and because ρ is not sensitive to monotonic curvilinearity in the relationship. The data in Fig. 2A show a highly significant dependence of catch ratio on fish length ($\rho = 0.960$, $P < 0.01$).

The next phase of interpretation is to identify one or more theoretical Ralston Plots with shapes that might correspond to those derived from the field data. Figure 2A shows a slowly ascending limb followed by a rapid increase in slope at fish lengths above 40 cm. (The rightmost point was based on the capture of 11 fish and was followed by two further points, of indeterminate catch ratio, at fish lengths where the larger hooks took single fish and the smaller ones took none.) Thus, I suggest that the figure is consistent with normal selection, if (1) the optimal lengths of the two hooks are close relative to their selection ranges, which might be expected with the weak selection often claimed for baited hooks, and (2) the sizes of fish available were mostly less than the optimal length of the larger hook (thus holding the catch ratios to values around 1; see Fig. 1Dii). These data might also be fitted by skew normal or gamma selection curves, although the additional parameters seem unlikely to be necessary. It is improbable that an asymptotic function would yield as good a fit. Figure 2A therefore indicates that the normal curve would give an appropriate functional form when beginning nonlinear estimation of the selectivities of these hooks. It also suggests, however, that the data set may contain too few catches of large fish for the true form of the selection curve to be determined reliably.

The catch ratios from Ralston's (1990) data on *Pristipomoides auricilla* are shown in Fig. 2B, again with the addition of some points excluded from his published plot. These data show a much weaker, but still marginally significant, link between catch ratio and fish length ($\rho = 0.606$, $P < 0.05$) because of the anomalous rightmost point. That point could, perhaps, be dismissed as an artifact of the complex error structure (it is based on a single fish and not supported by data on any other fish lengths), raising ρ to 0.989 and leaving the plot as a rather narrow, inflected curve. That is the shape expected with skew normal selection if the optimal lengths for the two hook sizes are similar (Fig. 1Giii) or if they are indistinguishable while the selectivity functions for those sizes differ in their values of the skew parameter (Fig. 1Iiii). The optimal length would then correspond to the length at the point of inflexion of the Ralston Plot: between 275 and 375 mm fork length in this case. It may also be noted that the overall efficiency of the larger hooks must be only about one third that of the smaller ones, since the point of inflexion lies at a catch ratio of about 0.3. The two leftmost points in the plot are little better supported than the rightmost, however. Their deletion would leave a more generalized Ralston Plot that would be consistent with most forms of normal, skew normal, or gamma selection curves. Thus, Fig. 2B cannot conclusively demonstrate skew normal selection, but it does indicate that this would be an appropriate functional form for initial fitting, if the data set contains sufficient data on extreme fish sizes for the true form of the selectivity curve to be determined at all.

Cortez-Zaragoza et al. (1989) have published some data on comparative hand-lining for yellowfin tuna (*Thunnus albacares*) using nine different hook sizes. Their hooks of 12- to 16-mm gap (six different sizes) took fish 22–62 cm long, while those of 24- to 29-mm gap (three sizes) took fish 126–162 cm long. Thus, their work took the form of two discrete selection experiments. They fitted normal selection curves to their data, using Holt's (1963) method, on all possible pairs of adjacent hook sizes. This allowed them to show that selection range increased asymptotically with hook size (despite Holt's assumption that selection range is constant across the pair of gears compared), while optimum length increased without limit as hook size increased. Figure 2C is a Ralston Plot of their data from 13- and 14-mm-gap hooks, while Fig. 2D is based on their data for 13- and 16-mm hooks. These plots are the clearest of the 21 possible from their data but are representative of most of the rest, the residue being apparently badly affected by the inevitable measurement error.

Both Fig. 2C and 2D show highly significant relationships between catch ratio and fish size ($\rho = 0.985$, $P < 0.01$; $\rho = 0.912$, $P < 0.01$, respectively) and, in shape, are consistent with normal selection and rather wide-set optimal lengths (relative to selection range). The latter conclusion is in accord with the lack of overlap in the lengths of fish caught by the 16- and 24-mm hooks, although it is rather surprizing in light of the generally weak selection noted in other studies of hooks. These plots might also be consistent with skew normal or gamma selection, but any attempt to fit asymptotic selectivity functions would require improbably large efficiency differences between the various hook sizes.

Takeuchi and Koike (1969) carried out comparative trials of three sizes of each of two hook types when longlining for yellowfin goby (*Acanthogobius flavimanus*). A Ralston Plot of their data on No. 13 and No. 5 Ryusen–Sodegata hooks (Fig. 2E; $\rho = 0.976$, $P < 0.01$) suggests that the selection curves may follow the gamma function with a low value of α and very close-set optimal lengths, relative to the selection range (cf. Fig. 1Jii). This plot could also be consistent with the curves being skew normal and showing a difference in skew between the hook sizes but little difference in optimum length, if it were assumed that no fish larger than the common optimal length were taken (cf. Fig. 1Giii). Provided that the aim is to fit a curve to the data, rather than to extrapolate that curve to fish sizes for which no data are available, the gamma function would be preferable in this case as being more flexible in fitting. The Ralston Plot suggests that the data set is insufficient for any more ambitious aim.

Finally, McCracken (1963) attempted comparative longlining experiments for Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) using three sizes of hooks (Nos. 17, 14, and 11). His haddock data showed no recognizable selective effect, but he concluded that the selection for Atlantic cod was asymptotic, apparently because examination of length frequencies indicated that all hook sizes took similar numbers of large fish. Unfortunately, McCracken's full data set is not available, but Koike et al. (1968) have published some of his data on Atlantic cod, with the tails of the catch-at-length distribution deleted. When these data are recast as Ralston Plots (Fig. 2F, 2G; $\rho = 0.900$, $P < 0.01$; $\rho = 0.973$, $P < 0.01$, respectively), they can be seen to be not inconsistent with logistic selection, if appropriate points were rejected as artifacts of sampling error. They are, however, much more suggestive of normal selection, the No. 11 hooks particularly taking considerably more large fish than the No. 17 hooks. Once again, McCracken's data set appears insufficient for a confident determination of the shape of the selectivity curves of his hooks, but Fig. 2F and 2G do provide a clear indication that some form

of domed function would be more appropriate than any asymptotic one.

Discussion

In contrast with Ralston's (1990) lack of success and despite the low quality of the available data sets, these examples do show that Ralston Plots prepared from field data can accord with theoretical expectations. They also show that these plots can give some preliminary indication of the functional form of the selectivity of baited hooks, although with the present data the fits of multiple functional forms would still need to be compared statistically to determine which was best. Indeed, despite their deficiencies, McCracken's (1963) data on Atlantic cod, which have been interpreted as the principal evidence for asymptotic selection by hooks, are shown in Fig. 2 to be more consistent with some form of domed selection. Thus, Ralston Plots show promise of being a valuable, as well as a rapid, precursor to the quantitative estimation of selectivities.

The impossibility of deriving firmer conclusions about the functional form of selectivity from the Ralston Plots in Fig. 2 suggests that the available data sets are inadequate. Only if sufficient fish of extreme lengths have been caught could Kirkwood and Walker's (1986) nonlinear analytical approach distinguish between, for example, normal and skew normal selection. Ralston Plots offer a convenient way to determine, while still engaged in the fieldwork, what length ranges are "extreme" for a particular experiment and whether sufficient data have been gathered for the catch ratios to be mutually consistent across those ranges.

Besides their use as precursors for statistical analysis, Ralston Plots may have some potential, in situations where powerful computational facilities are not available, as a tool for the final analysis of selectivity data or as a guide to which of the manual fitting methods is most appropriate. These plots could be seriously misleading when so used, however, inasmuch as arbitrary discarding of points and subjective curve fitting could allow the investigator to adapt his interpretation to conform to almost any preconceived selectivity function. Thus, I recommend that Ralson Plots only be used as stand-alone analytical tools if they show an unambiguous curve, identifiable to a single form of selectivity function and in which each of the points is based on catches of many fish. Such a plot would require a much larger data set than would be needed for statistical analysis.

Whether Ralston Plots are used alone or are followed by nonlinear estimation, the resulting models of selectivity will be purely descriptive and will relate only to the data sets analyzed. These approaches are, therefore, as equally applicable to studies of the selective effects of different bait sizes or variations in other aspects of the gear as they are to studies of different hook sizes, provided that the selectivities of both experimental gears follow the same functional form and differ only in parameter values. It will remain for the investigator to design his experiment so that the gears differ only in the one attribute of interest.

Finally, it should be remembered that the range of selectivity functions illustrated here does not exhaust all possibilities, although it does include those frequently used in past studies. Users of Ralston Plots are, therefore, encouraged to investigate other functional forms that might better fit their data and to examine the shape of the resulting plots.

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Interactive Influence of Turbidity and Light on Larval Bluegill (*Lepomis macrochirus*) Foraging

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Miner, J.G., and R.A. Stein. 1993. Interactive influence of turbidity and light on larval bluegill (*Lepomis macrochirus*) foraging. Can. J. Fish. Aquat. Sci. 50: 781–788.

In a series of in situ enclosure experiments with larval bluegill (*Lepomis macrochirus*), we demonstrate that turbidity from suspended sediments reduces bluegill consumption of crustacean zooplankton, primarily cyclopoid copepods and copepod nauplii. However, this reduction occurred only when light intensity in parts of enclosures fell below a threshold, estimated at <450 lx. Following recent studies demonstrating copepod diel vertical migration in response to predators, it appears that copepods in our experiments used low-light strata as a refuge. Without this apparent refuge present, larval bluegill consumption increased with increasing turbidity, but prey were smaller on average. Thus, prey biomass consumed by larval bluegill did not differ with turbidity in high-light conditions. We postulate that the shift to smaller prey across taxa at higher turbidity, when light intensity exceeded 450 lx, derives from increased prey–background contrast. In low-light conditions, larval bluegill consumed larger, but fewer, zooplankton with increasing turbidity, resulting in lower prey biomass consumed. Thus, we demonstrate the field conditions causing negative turbidity effects on larval fish foraging success, and thus growth and recruitment.

Dans une série d'expériences in situ (dans une enceinte) avec des larves de crapet arlequin (*Lepomis macrochirus*), nous avons démontré que la turbidité provoquée par les sédiments en suspension réduisait la consommation de crustacés zooplanctoniques, surtout des copépodes cyclopïdes et des nauplii de copépodes. Toutefois, cette réduction ne s'observait que lorsque l'intensité lumineuse dans certaines partie de l'enceinte était inférieure à une valeur seuil estimée à <450 lx. Des études de suivi récentes ayant montré la migration verticale nycthéméral des copépodes en réponse à l'action des prédateurs, il semble que dans nos expériences les copépodes aient utilisé les couches de faible intensité lumineuse comme refuge. Sans la présence de ce refuge apparent, la consommation des crapets larvaires augmentait avec l'accroissement de la turbidité, mais les proies étaient en moyenne plus petites. Ainsi, la biomasse des proies consommées par les crapets larvaires n'a pas changé avec la turbidité dans des conditions d'intensité lumineuse élevée. Nous avons postulé que le changement vers la consommation de proies plus petites dans l'ensemble des taxons en présence d'une turbidité plus élevée, lorsque l'intensité lumineuse dépasse 450 lx, est attribuable une augmentation du contraste entre les proies et le fond. Dans des conditions de faible luminosité, les crapets larvaires ont consommé du zooplancton moins nombreux, mais plus gros, au fur et à mesure qu'augmentait la turbidité, avec le résultat que la biomasse des proies consommées est plus petite. Ainsi, nous avons démontré les conditions qui, sur le terrain, sont à l'origine des effets négatifs liés à la turbidité sur l'alimentation des poissons larvaires et, par conséquent, sur la croissance et le recrutement.

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Survival of larval fish largely depends on size-selective predation (see reviews in Blaxter 1986; Miller et al. 1988). Therefore, any environmental factors affecting larval growth or exposure to predation should influence recruitment success (Miller et al. 1988; Margulies 1990). Turbidity from suspended sediments is a common feature of many lakes and reservoirs, particularly those in agricultural watersheds, and, along with sedimentation, is a major factor in rivers and lakes that can affect organisms throughout the aquatic community (see reviews in Muncy et al. 1979; Arruda et al. 1983; Wilber 1982; Bruton 1985; Goldsborough and Kemp 1988). Herein, we quantify how turbidity from suspended sediments influences foraging success by larval fish, interpreting these results in the context of larval growth, predation risk, and recruitment.

For particulate feeding juvenile and adult fishes, turbidity reduces foraging ability by reducing reaction distance (i.e., maximum distance for detecting prey and predators, Vinyard and O'Brien 1976; Crowl 1989). However, studies with larval fishes have produced conflicting results; turbidity has decreased, increased, and had no effect on foraging success or growth rate (Johnston and Wildish 1982; Boehlert and Morgan 1985; Breitburg 1988; Chesney 1989).

In studies where foraging rate by larval fish declined with increasing turbidity, results were postulated to occur because of reduced reaction distances (Johnston and Wildish 1982; Breitburg 1988), as has been demonstrated for adult fishes (Vinyard and O'Brien 1976; Barrett et al. 1992). Conversely, when turbidity enhanced foraging success of larval fishes,

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increasing contrast between prey and background has been suggested as the mechanism (Boehlert and Morgan 1985). Finally, the lack of a turbidity effect on larval striped bass (*Morone saxatilis*) growth (Chesney 1989), as turbidity increased from 0 to 130 nephelometric turbidity units (NTU; APHA et al. 1985), a typical range in estuaries and turbid reservoirs, suggests that other interacting variables affect foraging success.

Light intensity reduces foraging rate and growth of larval fish at levels <100 lx, although the rate of decline and exact light conditions causing the decline vary among species (see review in Blaxter 1986). For striped bass larvae, growth in clear water declines exponentially as light intensity falls from 300 to 0.4 lx, while growth does not change above 450 lx (Chesney 1989). When turbidity negatively affects foraging (Johnston and Wildish 1982; Breitburg 1988), light intensity above laboratory containers has been <300 lx and as low as 5 lx. Positive effects of turbidity on larval fish feeding occurred when light intensity was >800 lx (Boehlert and Morgan 1985).

Hence, increased turbidity in lakes and rivers may influence larval fish feeding rates primarily through increased light attenuation, which may result in suboptimal light conditions for foraging. In addition, turbidity can alter prey–background contrast (Hinshaw 1985), as well as affect vertical distribution of fish larvae (Swenson and Matson 1976; Matthews 1984) and zooplankton (Zettler and Carter 1986). Given that light intensity declines with depth, results from experiments in shallow aquaria may not accurately reflect effects of turbidity on larval fish foraging in the field where predator and zooplankton prey can select optimal depths. Therefore, we designed field enclosure experiments using larval bluegill (*Lepomis macrochirus*), a common limnetic species that feeds on zooplankton (Werner 1967; Werner and Hall 1988; DeVries et al. 1991), to determine how turbidity influences its foraging in situ.

Methods

Foraging experiments were conducted on each of four consecutive days in Stonelick Lake, a 69-ha reservoir in southwest Ohio with an upstream-to-downstream turbidity gradient reflected by typical Secchi depths of about 20–120 cm, respectively. For these experiments, we placed two transparent polyethylene enclosures (1-m diameter) at each of three sites along the gradient. Depth was 1.7, 1.5, and 1.1 m at the low-, intermediate-, and high-turbidity sites, respectively; enclosures extended through the entire water column, but were closed at the bottom, excluding the mud substrate. Turbidity was ambient in the two lower turbidity treatments (i.e., about 10 and 20 NTU, Sargent-Welch turbidimeter). To attain the high-turbidity treatment of about 30–40 NTU, we added small quantities of bottom sediments (i.e., from 50-cm sediment cores at the high-turbidity site) to enclosures the day before an experiment began. However, during one set of experiments, too much sediment was added to one enclosure resulting in a turbidity of 64 NTU.

Enclosures were filled 1 d before an experiment with filtered (60-μm mesh) water pumped from a depth of 1 m. To generate similar zooplankton assemblages among turbidities, organisms for all enclosures were collected at the intermediate-turbidity site. Water was pumped from a depth of 1 m and two times the volume of a single enclosure filtered through a 60-μm-mesh net. After thorough mixing, the zooplankton sample was split equally and half was introduced into each of the two enclosures within a turbidity treatment. Because of travel time between turbidity treatment sites, we did not completely randomize inoculations of zooplankton among enclosures.

Larval bluegill (12.5 ± 0.08 mm total length, $\bar{X}$ ± SE) were collected from a nearby pond, held in the laboratory for at least 48 h, and fed *Artemia* sp. nauplii and a mixed culture of zooplankton (mostly copepods) daily. Larvae were then starved for about 18 h before release into enclosures. We added 15, 13, and 10 bluegill to low-, intermediate-, and high-turbidity enclosures, respectively; this resulted in a consistent density of 11 larvae · m^{-3} among enclosures of different depths. Peak density of bluegill larvae in Stonelick Lake averages 2.2 fish · m^{-3} (DeVries et al. 1991).

Bluegill larvae were introduced into 3-L containers (with 500-μm-mesh sides), which were hung inside enclosures overnight. Before release of larvae into enclosures, water in each enclosure was mixed to resuspend sediments and zooplankton were sampled (i.e., after mixing). Water was not mixed during these short-term experiments because turbidity never declined sufficiently to cause turbidity in any treatments to overlap (i.e., maximum change at lowest and highest turbidity during experiments was about 2 and 10 NTU, respectively). Zooplankton were sampled with a 5.1-cm-diameter tube, collecting ≥5 L of water from the entire water column. Water was filtered through a 60-μm-mesh net and zooplankton were preserved in 10% formalin.

Bluegill larvae were released into enclosures at 9:00–10:00 and allowed to feed for 3.5–5.5 h, which ensured evacuation of initially ingested prey. Starved larvae select larger prey at first-feeding than are consumed during continuous feeding (Confer and O'Bryan 1989). We did not determine gut passage time explicitly for bluegill, but larvae of another centrarchid, largemouth bass (*Micropterus salmoides*), evacuate in about 2 h at 22–23°C (Laurence 1971). Water temperature in our enclosures was 24–27°C. Because evacuation rate increases with temperature, we believe that initially eaten prey had passed through larval guts within the duration of our experiments.

At experiment's end, turbidity was measured at the 0.5-m depth in all enclosures. In two sets, light intensity (measured as illuminance with a LICOR LI-188b photometer), dissolved oxygen, and temperature were measured at 0.5-m depth intervals in one enclosure for each turbidity treatment. Water was pumped out of enclosures (i.e., duration = ~10 min) and bluegill larvae were filtered from this water. Not all larvae were retrieved intact because of pump damage; those collected were preserved in 10% formalin.

To quantify zooplankton abundance and composition, we identified at least 200 crustacean zooplankters from each sample. These organisms were obtained by adding the entire sample to a graduated cylinder and then removing vertically well-mixed subsamples with a large-bore (8-mm-diameter) pipette. The first 10 individuals identified of each taxon per sample were measured for length (nearest 0.034 mm, excluding spines and caudal rami); these lengths were converted to biomass using length – dry weight regressions (Dumont et al. 1975; G.G. Mittelbach, Kellogg Biological Station, Michigan State University, East Lansing, Mich., unpublished data).

We identified prey to the lowest taxon possible (86% of cladocerans to genus and 98% of adult copepods to suborder) in guts of 5–15 bluegill larvae from each enclosure. Up to 10 prey·taxon^{-1}·fish^{-1} were measured for length to estimate biomass consumed. We used Chesson's alpha (Chesson 1978, 1983), which compares the proportion of each taxon in the diet and in zooplankton samples, to quantify selection of prey by

TABLE 1. Light intensity (lx) across turbidities in enclosures with foraging bluegill larvae during low and high ambient light in Stonelick Lake, Ohio

	Low light (set 3) at turbidity (NTU):				High light (set 4) at turbidity (NTU):		
Depth (m)	11.5	18	43	64	10.5	19.5	31
0.0	7540	8400	4080	5020	63000	57000	65400
0.5	1960	1590	210	180	21400	10000	6980
1.0	457	314	14	5	5500	1200	479

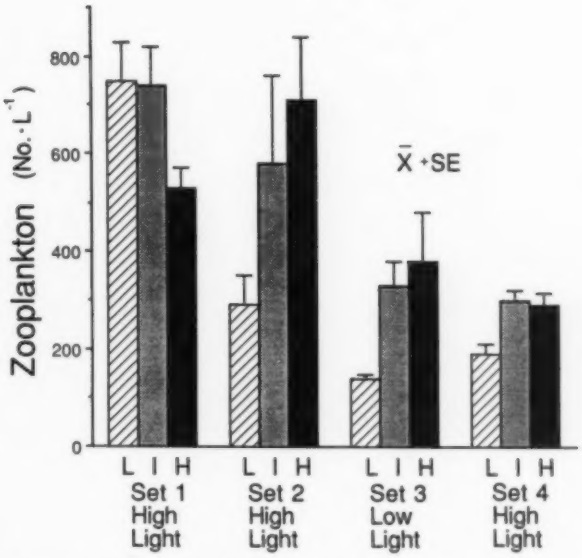

FIG. 1. Zooplankon density in enclosures with varying turbidity in Stonelick Lake, Ohio, on four consecutive days. Enclosures were placed at low (L), intermediate (I), and high (H) turbidity sites within the lake. Standard errors are based on a single sample from each of two enclosures at each site.

larval bluegill. Because enclosures were the experimental units, proportions in the diet for alpha calculation were determined by pooling numbers consumed by all fish within each enclosure, rather than calculating alpha for individual fish and averaging for an enclosure (see Cochran (1977) for a discussion of averages of ratios). The formula for this index is

$$\text{alpha} = \frac{r_i/p_i}{\Sigma\,(r_i/p_i)}$$

where the proportion of prey item i is p_i for the environment (enclosures) and r_i for the diet. The index ranges from zero to 1, with zero indicating complete avoidance of the prey taxon.

To determine if initial zooplankton densities in enclosures differed among turbidity treatments (sites) and sets (dates), we conducted two-way ANOVA of total zooplankton density (log transformed). There was significant interaction between treatments and sets ($F = 3.3$; df = 6,12; $p = 0.037$), suggesting patchy zooplankton distribution (Fig. 1). However, the range of zooplankton density was similar among turbidity treatments over the course of the experiment; therefore, we analyzed data for prey consumption as a function of turbidity, using multiple linear regression analysis, including zooplankton density as an independent variable.

Results

Light intensity was high for sets 1, 2, and 4 because there was little or no cloud cover. Overcast conditions prevailed during set 3 such that surface light intensity was reduced by 90% relative to set 4 (Table 1). Light intensity was not measured in sets 1 and 2, although weather conditions were similar to those in set 4. At 1-m depth in set 3, light intensity ranged between 5 and 460 lx from the highest (64 NTU) to lowest (11 NTU) turbidity (illuminance measured before an enclosure was drained) whereas in high-light conditions, light intensity remained >460 lx for all turbidities and depths (Table 1). Because low light (<100–300 lx) influences larval fish feeding and growth (Blaxter 1986; Chesney 1989), we analyzed high-light and low-light data separately.

Abundance in Diet

Number of zooplankton consumed by bluegill larvae was correlated with turbidity, but responses differed with light intensity (Fig. 2). At high light, larvae ate more zooplankton as turbidity increased ($r^2 = 0.35$; $p = 0.006$; $N = 18$), while fewer zooplankton were consumed with increasing turbidity at low light ($r^2 = 0.86$; $p = 0.005$; $N = 6$). Regardless of light intensity, neither zooplankton density nor the interaction between turbidity and density improved the fit of these models ($p = 0.51$ and 0.79, respectively, at high light and $p = 0.48$ and 0.37, respectively, at low light from multiple linear regressions). No zooplankton density effect was detectible, even though there was a wide range of density among enclosures (i.e., 125–800 organisms·L^{-1}).

Among prey groups, cyclopoid copepods and copepod nauplii were selected by bluegill larvae, while copepod nauplii and the cladocerans *Bosmina longirostris* and *Ceriodaphnia* sp. were the most common prey in the environment (Fig. 3). Cyclopoid copepods and copepod nauplii consistently composed about 70% of prey consumed.

Because high and low light intensities occurred during different sets, differences attributed to light intensity – turbidity interactions may have derived from date effects. However, other variables potentially affecting larval bluegill foraging, such as dissolved oxygen concentration and temperature, were consistent during this 4-d experiment (Table 2). Hence, we conclude that larval bluegill foraging was influenced by light intensity as mediated through turbidity.

Prey Selection

To determine if turbidity affected prey selection, we regressed Chesson's alpha for each taxon against turbidity at high and low light. If turbidity failed to modify prey selection by larvae (t-test of slope, $p > 0.05$), we compared mean selectivity across turbidities to an expected value based on neutral

TABLE 2. Physical conditions in enclosures during larval bluegill foraging trials in Stonelick Lake, Ohio, June 27–30, 1988. Turbidity, temperature, and dissolved oxygen data were taken at 0.5 m.

	Day			
	1	2	3	4
Turbidity (NTU)				
Low	9, 10	8, 9	11, 11.5	10, 10.5
Intermediate	16.5, 17	17.5, 18	18, 19.5	19, 19.5
High	29, 29	39, 39.5	43, 64	31, 31
Temperature (°C)	25.5–27	24	25	24–26
Oxygen ($mg \cdot L^{-1}$)	9.9–11.6	—	—	8.6–9.8

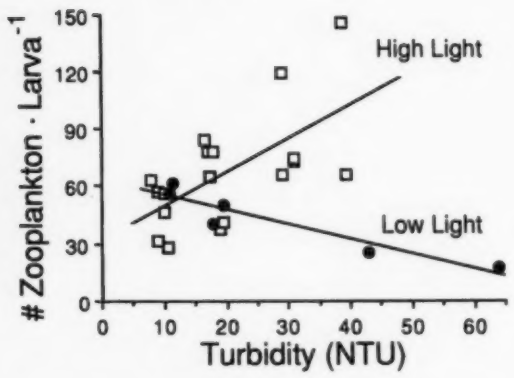

FIG. 2. Mean number of zooplankton consumed per larval bluegill from each enclosure in high-light (cloudless) and low-light (overcast) treatments in Stonelick Lake, Ohio. Each symbol represents a mean per enclosure (N = 5–15 per enclosure).

selection (i.e., the reciprocal of the number of prey taxa in the environment, which in this case was alpha = 0.14).

At high light, only selection for copepod nauplii and calanoid copepods changed as a function of turbidity (Table 3). Copepod nauplii were negatively selected at low turbidity (alpha = 0.02 ± 0.01; $\bar{X}$ ± SE). However, selection for copepod nauplii increased with increasing turbidity, resulting in positive selection at the highest turbidity (alpha = 0.33 ± 0.10; Fig. 3). In contrast, as turbidity increased, selection for calanoid copepods declined, and was always less than neutral selection (alpha = 0.05 ± 0.02 at about 10 NTU; Table 3). Although selection for other taxa was unaffected by turbidity at high light, cyclopoid copepods and *Diaphanosoma leuchtenbergianum* were consistently preferred by larval bluegill (alpha = 0.43 ± 0.04 and 0.32 ± 0.05, respectively); conversely, *B. longirostris*, *Ceriodaphnia* sp., and *Daphnia parvula* were negatively selected (alpha ≤ 0.05; Table 3).

Low light occurred during only one set of experiments; thus, sample size (N = 6) and the power to detect changes in selection were low. Given this caveat, prey selection did not change across turbidity for any prey at low light intensity ($p > 0.05$; Table 3). As in high light, cyclopoid copepods were positively selected at all turbidities (alpha = 0.62 ± 0.05; Table 3) whereas small cladocerans (i.e. *B. longirostris* and *Ceriodaphnia* sp.), calanoid copepods, and copepod nauplii were negatively selected (Table 3).

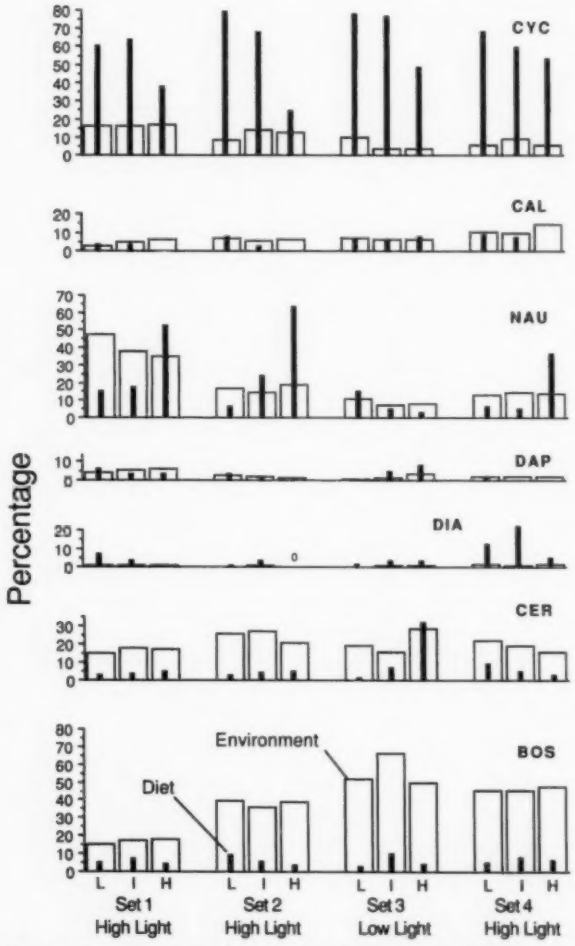

FIG. 3. Proportional abundance of zooplankton in enclosures and in diet of larval bluegill in Stonelick Lake, Ohio, at low (L), intermediate (I), and high (H) turbidity. CYC, cyclopoid copepods; CAL, calanoid copepods; NAU, copepod nauplii; DAP, *Daphnia parvula*; DIA, *Diaphanosoma leuchtenbergianum*; CER, *Ceriodaphnia* sp.; BOS, *Bosmina longirostris*.

Size Selection

Mean length (log transformed) of zooplankters consumed by larval bluegill declined as turbidity increased at high light intensity, corresponding to the increased selection for copepod nau-

TABLE 3. Regression analysis of prey selection (using Chesson's alpha, Chesson 1978, 1983) by larval bluegill in enclosures in Stonelick Lake, Ohio, as a function of turbidity at high-light (cloudless) and low-light (overcast) intensity. When slopes did not differ from zero (t-test, $p < 0.05$), mean alphas were compared with neutral selection (i.e., 0.14, reciprocal of the number of prey taxa in the environment). A dash indicates that the mean was not calculated because turbidity affected prey selection by larvae.

	High light (N = 18)						Low light (N = 6)					
	H_0: slope = 0			H_0: mean = 0.14			H_0: slope = 0			H_0: mean = 0.14		
Prey taxa	t	$p > t$	r^2	alpha ± SE	t	$p > t$	t	$p > t$	r^2	alpha ± SE	t	$p > t$
Copepods												
Nauplii	4.91	<0.001	0.60	—	—	—	−1.79	0.15	0.45	0.04 ± 0.02	−5.37	<0.01
Calanoid	−2.90	0.01	0.34	—	—	—	0.42	0.70	0.04	0.05 ± 0.01	−6.68	<0.01
Cyclopoid	−0.79	0.44	0.04	0.43 ± 0.04	6.50	<0.001	−0.31	0.77	0.02	0.62 ± 0.05	10.40	<0.01
Cladocerans												
Bosmina longirostris	−0.42	0.68	0.01	0.01 ± 0.00	−51.13	<0.001	−0.63	0.56	0.09	0.00 ± 0.00	−156.3	<0.001
Ceriodaphnia sp.	1.90	0.08	0.18	0.01 ± 0.00	−85.71	<0.001	1.80	0.15	0.45	0.02 ± 0.01	−12.43	<0.001
Daphnia parvula	−1.36	0.19	0.10	0.05 ± 0.01	−7.05	<0.001	−0.75	0.49	0.12	0.10 ± 0.03	−1.31	>0.2
Diaphanosoma leuchtenbergianum	−1.62	0.13	0.14	0.32 ± 0.05	3.62	<0.01	2.13	0.10	0.53	0.16 ± 0.03	0.58	>0.5

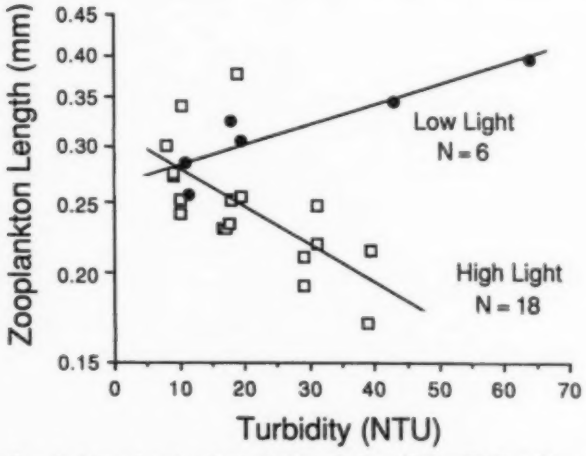

FIG. 4. Mean length of zooplankton in larval bluegill diets from enclosures along a turbidity gradient in Stonelick Lake, Ohio. See Table 1 for differences between light conditions. In high light, r^2 = 0.43 and p = 0.003; in low light, r^2 = 0.83 and p = 0.012.

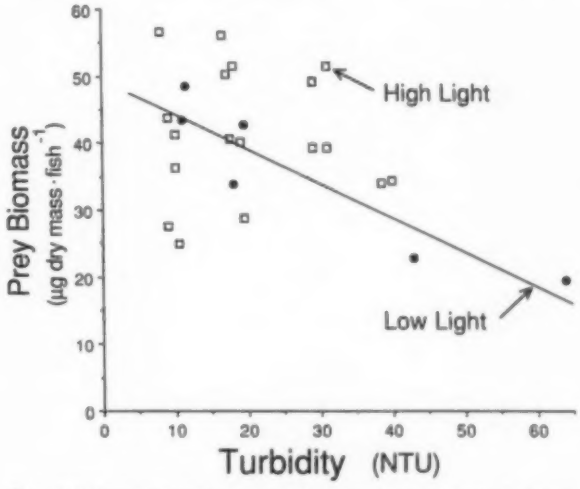

FIG. 5. Biomass of prey recovered from larval bluegill guts as a function of turbidity at high and low light in Stonelick Lake, Ohio.

plii (r^2 = 0.43; p = 0.003; N = 18; Fig. 4). At 10 NTU, predicted mean prey length was 0.28 mm whereas mean length declined 30% to 0.19 mm at 40 NTU. When we compared prey length within a taxon, smaller cyclopoid copepods and copepod nauplii (i.e., composing 59 and 22% of prey in the diet across all turbidities) were consumed by larval bluegill as turbidity increased at high light (F = 3.61; df = 4,14; p = 0.03; MANOVA using Wilk's criterion, SAS Institute, Inc., 1985).

At low light intensity, bluegill consumed larger prey as turbidity increased (r^2 = 0.83; p = 0.012; N = 6; Fig. 4). Mean prey length at low turbidity (11 NTU) was 0.28 mm, the same as at high light. However, at high turbidity (43 and 64 NTU), larvae consumed prey that averaged 0.35 mm long, representing a 75% increase in mean prey length over that consumed in high-light sets at high turbidity. Also, at low light, size of cyclopoid copepods and copepod nauplii consumed increased from low to high turbidity (F = 9.17; df = 4,4; p = 0.02; MANOVA). In summary, at high light, bluegill larvae tended to select smaller zooplankton with increasing turbidity whereas prey size increased when turbidity increased at low light.

Biomass Consumed

Because number of prey consumed increased and size of prey decreased with turbidity at high light, and because reciprocal results were obtained at low light, we compared dry mass of zooplankton consumed across turbidity. At high light, the increase in number of prey consumed was offset by a reduction in prey size, resulting in no significant relationship between turbidity and biomass consumed (r^2 = 0.00; p = 0.83, N = 18; Fig. 5). However, at low light, biomass consumed declined with increasing turbidity (r^2 = 0.85; p = 0.009; N = 6; Fig. 5). Therefore, whereas turbidity influenced selection of prey taxa consumed by larval bluegill, change in prey biomass consumed was detected only for the low-light condition.

Discussion

Foraging by larval bluegill was influenced by interaction between turbidity and light. When light intensity throughout enclosures exceeded 450 lx, increasing turbidity caused larvae

to select smaller prey both within and among taxa. However, biomass of prey consumed did not differ across turbidities because numbers of prey increased with turbidity. Conversely, when light intensity in large portions of enclosures fell below 300 lx during low ambient light conditions (i.e., set 3), both prey numbers and biomass consumed declined with increasing turbidity. These data provide insight into conflicting results in the literature for larval fish in which turbidity effects were measured at only one light intensity (i.e., either low or high light, Johnston and Wildish 1982; Boehlert and Morgan 1985; Breitburg 1988).

Johnston and Wildish (1982) found that larval Atlantic herring (*Clupea harengus*) consumed fewer prey as turbidity was increased from 0 to about 13 NTU at 300 lx (i.e., 20 mg estuarine sediments·L^{-1} calculated from Breitburg 1988), while larval Pacific herring (*Clupea pallasi*) consumed more prey at 90 NTU than in clear water when light intensity was >800 lx (Boehlhert and Morgan 1985). Striped bass larvae also consumed fewer prey organisms from natural assemblages of copepods as turbidity increased at low light (i.e., 5 lx; Breitburg 1988). Our field results support these observations, in which turbidity has negative effects on foraging only at low light, and may enhance prey contrast at higher light intensity, increasing prey detection and consumption by larvae (see Hinshaw 1985 for discussion of contrast).

Larval fish responses to turbidity have been explained in terms of reduced reaction distance with increasing turbidity, as occurs with juvenile and adult particle-feeding planktivores (Howick and O'Brien 1983; O'Brien 1987). However, relative to adults and juveniles, larval bluegill have poor visual acuity. Although bluegill larvae rapidly develop a complex visual system as they grow (Kawamura and Mukai 1984), reaction distance (i.e., acuity) is probably little more than one body length at a size of 12 mm total length (TL) (review for other species in Blaxter 1986; Wanzenbock and Schiemer 1989). In contrast, when bluegill are 31 mm TL, they possess retinas with a high density of rods and cones (Hairston et al. 1982; Breck and Gitter 1983), enabling them to detect zooplankton as far as 30 cm away (Vinyard and O'Brien 1976). When reaction distance is short, there are fewer suspended particles between predator and prey to reflect light and interfere with detection (i.e., much as fog affects long-distance vision, but has little effect on detection of close objects). Thus, while juvenile and adult bluegill exhibit an exponential decline in reaction distance with increasing turbidity (Vinyard and O'Brien 1976; Miner 1990), there may be little negative effect on reaction distance of larvae with sufficient light (>300 lx).

In contrast with the results at high light, increasing turbidity in the low-light set appears to have reduced light intensity below a threshold necessary for maximizing foraging rates by larval bluegill. Because larvae possess a rudimentary visual system with relatively few rods necessary for vision at low light intensity, feeding and growth can be negatively affected at low light (<100–300 lx, Blaxter 1986; Chesney 1989). In our experiments at low ambient light, high- and intermediate-turbidity enclosures contained extensive areas in which light intensity was <300 lx and prey consumption was depressed. However, in these in situ enclosures, why would foraging decline if larvae could use areas with optimal lighting (i.e., top 0.5 m)? These results suggest that larval bluegill were either using the low-light regions of enclosures causing foraging success to decline or zooplankton were concentrating in the low-light refuge, thus having the same effect on bluegill foraging.

Actively foraging larval fish can undertake relatively extensive diel vertical migrations (e.g., 10–30 m, Fortier and Leggett 1983; Wurtsbaugh and Neverman 1988; Munk et al. 1989) and appear to select optimal light levels. Increasing turbidity also tends to cause larval fish to migrate up in the water column, potentially to locate optimal light conditions for foraging (Swenson and Matson 1976; Matthews 1984; Boehlert and Morgan 1985). Because zooplankton also exhibit both diel vertical migration and migrate toward the surface as turbidity increases (Swenson and Matson 1976; Zettler and Carter 1986), the potential dissociation between prey concentrations and optimal search habitat for larval fish has not been considered. In experimental tanks, freshwater cyclopoid copepods tend to aggregate near the bottom even at night (Soto and Hurlbert 1991). Our data suggest that high turbidity and low light may create a refuge for zooplankton prey. The recent literature on diel vertical migration of zooplankton as a behavioral response to both invertebrate and vertebrate predators supports this refuge hypothesis (Ohman 1990; Ringelberg 1991; Bollens and Frost 1991).

Similar vertical distributions of larval herring and zooplankton, however, suggest synchronous habitat use (Fortier and Leggett 1983), although these observations were made over 10-m depth intervals. Because turbid conditions increase light attenuation rates, and thus compress light strata within shallow lakes (i.e., represented by our enclosures), zooplankton may concentrate within 50 cm of larval fish and still be within suboptimal habitat for fish foraging.

Zettler and Carter (1986) observed the progressive migration by zooplankters toward the surface along a turbidity gradient in a naturally turbid lake. In this deep lake (i.e., 50–60 m), they were able to show that many zooplankton concentrated in the top 5–10 m at high turbidity (14.5 NTU). However, using light attenuation rates generated from our system at 10.5 NTU in high light (see Table 1), light intensity at their high turbidity station was <300 lx at a depth of about 2.5 m. Thus, even with relatively fine-scale sampling (i.e., 5-m depth intervals in a system 60 m deep), in turbid systems it is difficult to determine if zooplankton concentrate within optimal habitat for larval fish foraging.

In high-light conditions, consumption of smaller zooplankton with increasing turbidity may be explained using predictions from optimal foraging theory (Stephens and Krebs 1986). Increasing turbidity at high light may increase prey contrast causing larvae to detect and consume smaller, and potentially more profitable, prey. Large prey are not always the most profitable in terms of net energy gain and growth (Bence and Murdoch 1986; Confer and Lake 1987) owing to increased handling time, poor capture efficiency, reduced assimilation efficiency, or increased gut passage time, as has been shown for early juvenile yellow perch (*Perca flavescens*, 22–62 mm TL) (Mills et al. 1986; Confer and O'Bryan 1989; Confer et al. 1990). Selection by yellow perch for cyclopoid and calanoid copepods and small *Daphnia* increases as prey density increases in high light whereas selection for large *Daphnia* declines. In fact, yellow perch grow faster feeding on small copepods than on *Daphnia* (Confer and Lake 1987). However, energy ingested per unit time does not differ between yellow perch feeding on copepods or *Daphnia*, in spite of the fact that foraging costs should be higher for copepods given their greater escape ability (Drenner et al. 1978). Given this, the observed increase in selection for smaller prey items (i.e., for copepod nauplii and smaller prey within taxa), and the fact that biomass consumed

did not change at high light, suggests that turbidity effects on larval fish are complex.

Results from this experiment point to strong interactions between turbidity and light intensity on larval fish foraging and help to explain conflicting results from previous laboratory studies. Rather than directly reducing foraging ability of larval fish by decreasing reaction distance, turbidity appears to affect larval foraging through a reduction in light intensity and possibly prey contrast. At low light, increasing turbidity causes a reduction in biomass of prey consumed by larval bluegill; however, even at these low-light conditions, optimal light intensity (>450 lx) occurred in the upper 0.5 m of each enclosure. Thus, the precise mechanism explaining reduced consumption of prey needs to be determined by quantifying larval bluegill and zooplankton vertical distribution and prey consumption along the vertical, low-light gradient. However, previous laboratory studies and our field experiment clearly demonstrate that explanations for larval fish responses to perturbations (e.g., increasing turbidity in coral reef communities, Roberts 1991) cannot be directly extrapolated from studies of juvenile and adult fish. Because of rapid ontogenetic changes, larval fish cannot be considered miniature adults.

When turbidity reduces light intensity to threshold levels (<450 lx in our system), spatial overlap between predator and prey or prey-capture abilities appears to be reduced in shallow lakes, and larval bluegill growth may be inhibited. Because of highly size-dependent mortality from predation (Margulies 1990), even minor reductions in growth rate can have substantial effects on survival through this critical life stage.

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Host Response of Atlantic Salmon (*Salmo salar*) to Infection by Sea Lice (*Caligus elongatus*)

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MacKinnon, B.M. 1993. Host response of Atlantic salmon (*Salmo salar*) to infection by sea lice (*Caligus elongatus*). Can. J. Fish. Aquat. Sci. 50: 789–792.

Atlantic salmon (*Salmo salar*), naturally infected with *Caligus elongatus* in seapen cages in Brandy Cove, St. Andrews, N.B., were examined histologically and ultrastructurally for signs of pathology associated with the attached chalimus larvae. Naturally -infected fish and fish that had never been exposed to sea lice infections were examined serologically for altered white blood cell counts and for any antibody response to the adult parasite. The chalimus larva feeds on the epidermal cells of the scale to which it is attached and denudes the epidermis down to the basement membrane. Ruptured and pycnotic cells line the hole created by the larva, and there is some evidence of hyperplasia around stage IV chalimus larvae. Little other damage or host reaction is evident. There is no seral antibody response to the patent infection as assayed by electroimmuno transfer blot, and white blood cell counts from infected and control groups of fish are statistically the same.

Des saumons de l'Atlantique (*Salmo salar*) élevés dans des cages flottantes situées à Brandy Cove, à St. Andrews (N.-B.) et naturellement parasités par *Caligus elongatus* ont fait l'objet d'un examen histologique et d'un examen de la structure cellulaire fine pour la recherche de symptômes d'un état pathologique lié à la présence des larves fixées. Des poissons infestés de façon naturelle et d'autres qui n'avaient jamais été exposés à des infestations par poux du poisson ont fait l'objet d'un examen sérologique pour la recherche d'un nombre anormalement bas de globules blancs ou d'une réaction immunitaire au parasite adulte. Les larves se nourrissent des cellules de l'épiderme des écailles auxquelles elles sont fixées et dénudent l'épiderme jusqu'à la membrane basale. Les dépressions faites par les larves étaient revêtues de cellules brisées ou pycnosées et l'on notait une certaine hyperplasie autour des larves de stade IV. Peu d'autres lésions ou réaction de l'hôte ont été notées. Il n'y avait pas de réaction sérique d'anticorps à une infection manifeste, telle que déterminée par buvardage, et les valeurs de dénombrement des globules blancs des groupes de poissons témoins et infectés étaient statistiquement les mêmes.

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Sea lice are ectoparasitic crustaceans that infect Atlantic salmon (*Salmo salar*). These parasites can be found in increasing numbers on fish that are stressed and unnaturally crowded such as those in seapen cages. Throughout the summer months, fish can be repeatedly infected with succeeding generations of sea lice and can acquire heavy infections by the autumn when water temperatures are highest. Both *Lepeophtheirus salmonis* and *Caligus elongatus* are present on salmon in the Bay of Fundy, but *C. elongatus* is by far the more common. The generation time of *C. elongatus* is approximately *49–81 d* depending on water temperature (Tully 1989) or 5 wk at 10°C as reported by Hogans and Trudeau (1989). The life cycle includes two free-swimming nauplius stages followed by the infective copepodid stage. The four chalimus stages are attached to fish scales by a frontal filament which anchors the larvae in one place throughout the interstadial moults. Two preadult stages and the adults move freely over the surface of the host. All stages on the host feed on mucus and epithelial cells but penetrate the dermis only in the case of very heavy infections.

The ability of sea lice to repeatedly infect the same fish suggests that Atlantic salmon have little effective immunity to these organisms. These preliminary investigations were designed to investigate the cellular response of mature *S. salar* to infection by the attached chalimus larvae of *C. elongatus*, to assay blood from infected and uninfected fish to determine if infected fish had an elevated leucocyte count, and to determine if any specific antibodies to adult sea lice were present in the serum of moderately infected (40–50 sea lice per host) fish.

Materials and Methods

Fish Stocks

One-sea-year Atlantic salmon, all of the same stock, that had been naturally exposed to sea lice infections for 1 yr provided experimental samples that were collected when water temperatures were 12°C. Similarly aged Atlantic salmon, from a different stock, that had been kept in artificial seawater at 12°C provided the control samples.

Cytology of Chalimus Larvae Attachment Sites

Five samples each of attached third- and fourth-stage chalimus larvae together with 1 cm^2 of tissue around each larvae were excised from freshly killed Atlantic salmon. Samples were fixed in Karnovsky's fixative, pH 7.2, for 24 h, rinsed 24 h in sodium cacodylate buffer, and postfixed 2 h in 1% osmium

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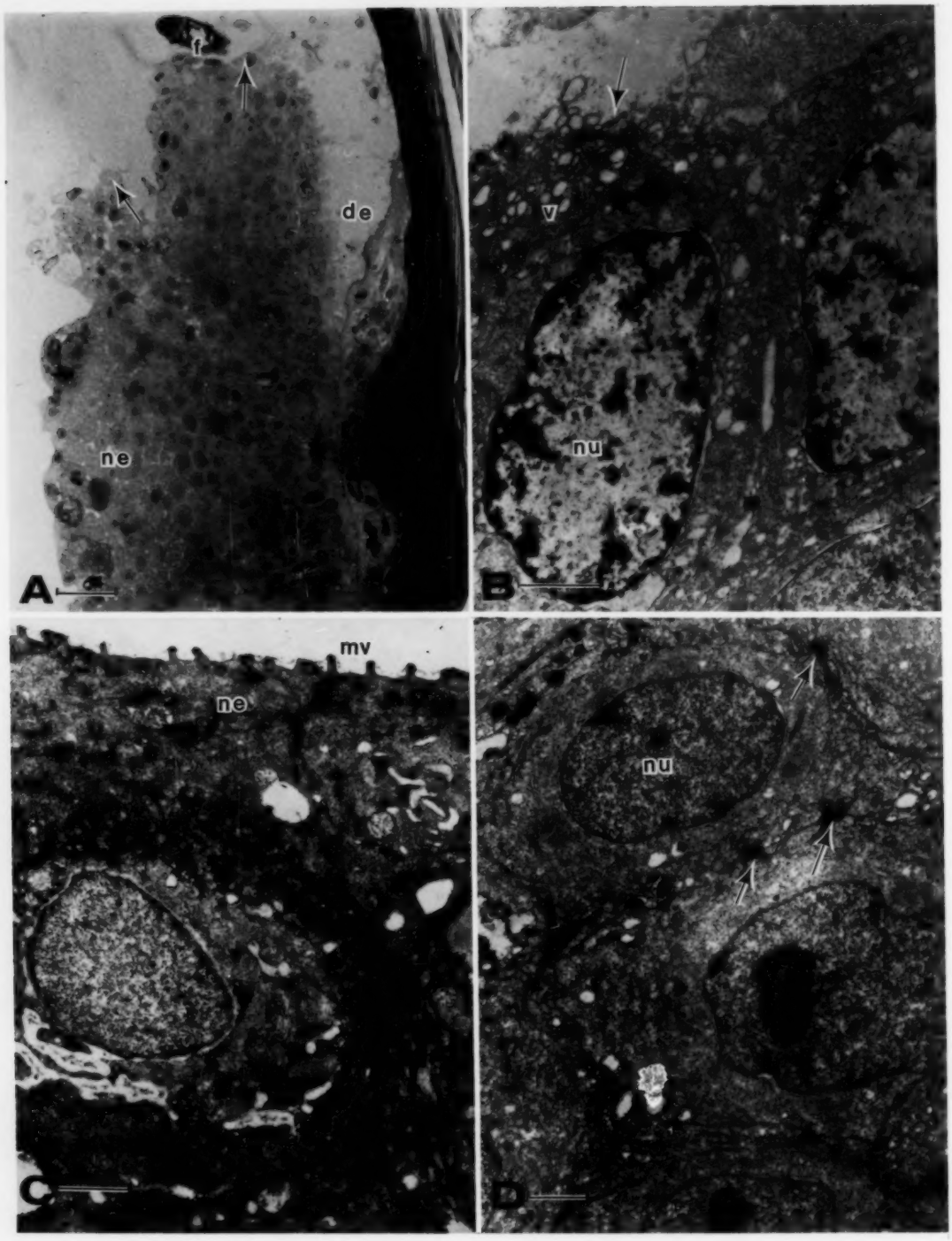

FIG. 1. (A) Light micrograph of a 1.0-μm section through the scale epidermis of *S. salar* infected with the chalimus larva of *C. elongatus*. f, frontal filament of the chalimus; arrows indicate area of tissue damage; de, detached epidermis; ne, normal epithelium. Bar = 30 μm. (B) Electron micrograph of the necrotic cells near the chalimus larva. v, vesicles characteristic of dying cells; arrow indicates area where plasma membrane is disrupted; nu, pycnotic nucleus. Bar = 2.0 μm. (C) Electron micrograph of normal epithelium adjacent to the necrotic area, showing the microvilli (mv) typical of healthy scale epithelium. Bar = 1.0 μm. (D) Electron micrograph of normal epithelial cells. Arrows indicate junctions between cells indicative of normal cell to cell movement of material. nu, nucleus. Bar = 1.0 μm.

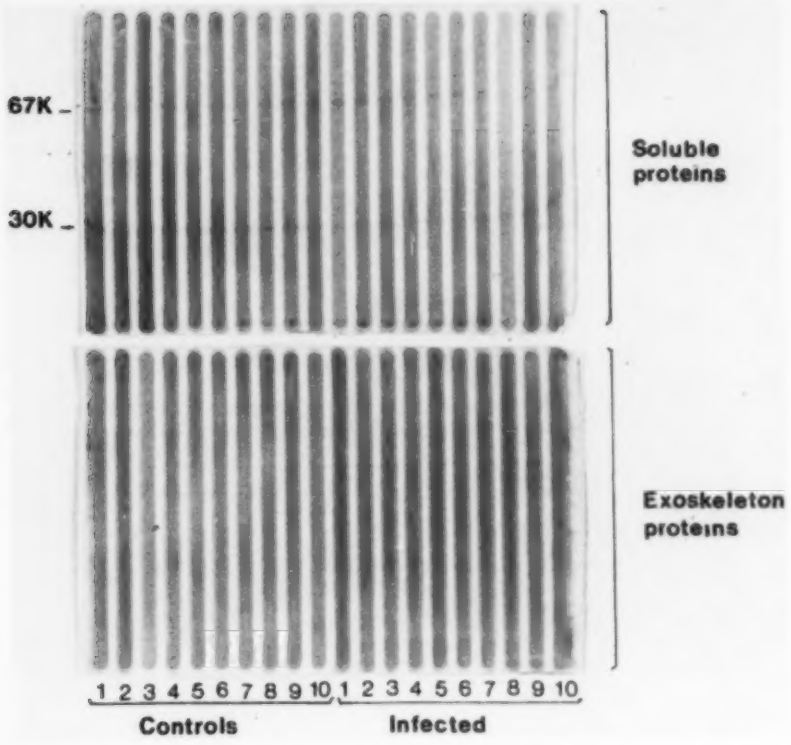

FIG. 2. Electroimmuno transfer blot of serum from uninfected and infected Atlantic salmon tested for the presence of specific antibodies to soluble and insoluble (exoskeleton) protein of *C. elongatus*. The faint lines at 30 and 67K and elsewhere are consistent between the two samples and may be considered standard reactions.

tetroxide. These were dehydrated in acetone and embedded in Epon 812 Araldite, and sections 1.0 μm and 60 nm thick were cut on an LKB microtome. Thick sections were stained with Toluidine blue and examined at the light microscope level, while thin sections were stained with uranyl acetate and lead citrate and examined ultrastructurally. Five tissue samples from uninfected fish were similarly examined.

Blood Samples

Blood samples were drawn from the caudal vein from 10 anaesthetized (MS 222) fish harbouring 40–50 parasites per fish and from 10 fish that had never been exposed to sea lice. Blood smears were made for each fish from infected and uninfected groups. These were stained in Wright's stain and random 1.0-mm^2 areas of cells, five areas per fish, were counted and the data for each fish pooled prior to statistical analysis using a Mann–Whitney test.

Specific Immunoglobulins

Serum was assayed for the presence of specific anti sea-lice antibodies by an electroimmuno transfer blot method (Tsang et al. 1983). Sea lice antigens were prepared from a crude homogenate of adult *C. elongatus* in phosphate buffer, pH 7.2. This homogenate was centrifuged at 9000*g* for 5 min and the supernatant used as the antigen source. Samples containing 70 μg of protein were run on an SDS–PAGE using a Mini Protean II electrophoresis apparatus (Bio-Rad) and transferred to nitrocellulose paper using an SD 250 transfer blot cell and a model 200/2.0 power supply (Bio-Rad) at 20 V for 30 min. The nitrocellulos blots were blocked for 30 min using 3% (w/v) gelatin in Tris-buffered saline containing 0.5% (v/v) Tween-20 (TTBS). The blots were transferred to a Mini Protean II multiscreen apparatus. The first binding reagent was a 2×10^{-2} dilution of the serum samples in TTBS containing 1% (w/v) bovine serum albumen, incubated for 18 h at 10°C, followed by six washes for 5 min with TTBS. The blots were developed using the second binding reagent, 2×10^{-4} rabbit anti-salmon Ig serum (made according to Olivier et al. 1992), incubated for 30 min, followed by two washes for 5 min with TTBS. The third binding reagent was a 1×10^{-4} dilution of donkey anti-rabbit Ig serum (Amersham) incubated for 30 min and followed by two washes for 5 min with TTBS. This procedure is further detailed in Olivier et al. (1992). Colour development of the blots are detailed in Griffiths et al. (1991).

Results

Chalimus larvae are anchored to the host scales by a frontal filament that penetrates through the epidermis and anchors into the basement membrane around the scale. Thus, all chalimus stages remain in one place feeding on the mucus and epidermal cells. The tissue surrounding the third- and fourth-stage chalimus larvae showed no tissue changes due to the presence of

the filament or any evidence of damage caused by the initial attachment by the second antennae of the copepodid. The tissue showed no infiltration by white blood cells. The feeding of the larvae resulted in excavation of a hole in the epidermis, down to the basement membrane, with detachment of the epidermal layer from the basement membrane in the area surrounding the hole in the majority of samples examined (Fig. 1A). Necrotic cells line the hole formed by the larva (Fig. 1B), while normal epithelial cells are present immediately surrounding the excavated area (Fig. 1C and 1D). Some samples of chalimus IV larvae showed evidence of slight hyperplasia around the excavated area. All control tissue samples showed intact normal epidermis.

Statistical (Mann–Whitney) analysis of the percent total white blood cells/erythrocytes as determined from blood smear counts for infected and noninfected salmon indicated no difference between the two groups ($p > 0.05$). However, average white blood cell counts from infected fish were lower than the average of those counts from uninfected fish.

Transfer blots of serum samples from infected and noninfected fish were identical (Fig. 2), suggesting that there are no specific antibodies to adult sea lice in Atlantic salmon blood when the fish harbour a moderate parasite burden.

Discussion

Third- and fourth-stage chalimus larvae were chosen for the investigation of host reaction to larval attachment and feeding, since it was felt that the nature of the tissue surrounding these larvae would reflect the cumulative effect of all previous chalimus stages. Except for the lesion caused by the feeding larva, there was little host response to the chalimus of *C. elongatus*. Chalimus larvae of *L. salmonis*, a less prevalent parasite in the Bay of Fundy, cause a more significant host reaction including mechanical disruption as a result of the feeding and attachment using the second antennae. In this latter species, a hyperplastic ring with associated melanization, fibroplasia, and macrophage infiltration is characteristic of attachment sites after the departure of the final chalimus stage (Jones et al. 1990). Similarly the attached stages of other caligids, *Lepeophtheirus pectoralis* and *Caligus uruguayensis*, cause fibroplasia and hyperplastic tissue growth (Boxshall 1977; Radhakrishnan and Nair 1981) with *L. pectoralis* causing increased infiltration by macrophages. Both *C. elongatus* and *L. salmonis* have similar modes and sites of feeding (Kabata 1974), and although *C. elongatus* has a greater variety and quantity of digestive system proteases (Ellis et al. 1990), *L. salmonis* is a larger and more aggressive feeder, causing more damage.

Moderate infections of *C. elongatus* do not cause open wounds on the fish, and thus it is perhaps not surprising that the host immune system does not "see" the parasite and does not produce specific antibodies. Interestingly, Atlantic salmon naturally infected with *L. salmonis* produce antibodies. One major antigen was detected in an unreduced sample of sea lice extract, and five antigens were detected when the sea lice samples were reduced with 2-mercaptoethanol (Grayson et al. 1991). These antigens were significantly fewer, however, than the 31+ antigens recognized by rainbow trout (*Oncorhynchus mykiss*) and rabbits immunized with sea lice extract (Grayson et al. 1991), indicating that natural infections do not present all possible antigens to the host.

Although the white blood cell counts from infected and control fish were not statistically different, the average blood cell counts from these preliminary data suggest that infected fish may have a lowered white blood cell count and warrant further investigation. Sea lice do stress fish and the resultant cortisol levels may lead to some immunosuppression. More extensive sampling and cortisol level analysis is in progress.

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Response of the Copepodid Larvae of *Caligus elongatus* to Light, and the Ultrastructure of the Eyes

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The copepodid larvae of *Caligus elongatus* are positively attracted to light over increasing light intensities using a vertical light source of 400 lx placed 25 cm from the larvae. The larvae are not attracted to the light source from a distance of 50 cm. Larvae in the dark did not migrate in any one particular direction. The copepodid larvae, examined ultrastructurally, have paired eyes with a triangular lensless third eye located below and between the two main eyes. The two main eyes each have an anterolaterally directed dorsal lens contained within a lens cell. Six rhabdomeric sensory cells and cup-shaped platelet sensory cells are contained by a pigment granule cup-shaped cell in each eye. Nerve axons from the sensory cells exit the eyes posterioventrally.

L'utilisation d'une source lumineuse de 400 lx placée à la verticale a permis de noter une attirance croissante en fonction de l'intensité lumineuse de larves de copépodes (*Caligus elongatus*) situées à 25 cm de la source. Les larves n'étaient pas attirées par la lumière lorsque la source était située à 50 cm et les larves dans l'obscurité ne se déplaçaient dans aucune direction particulière. L'examen au microscope électronique montre que les larves de copépodes possèdent des yeux pairs ainsi qu'un troisième oeil triangulaire médian postérieur sans cristallin. Les yeux pairs contiennent un cristallin dorsal en position antéro-latérale lui-même contenu dans une cellule cristallinienne. Six cellules sensibles à rhabdomes et des cellules sensibles à plaquettes ovées sont contenues dans un granule pigmentaire ové présent dans chaque oeil. Des axones reliés aux cellules sensorielles sortent de l'oeil en position postério-ventrale.

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Sea lice are ectoparasitic crustaceans that infect salmonid and other pelagic fishes. High numbers of sea lice can occur on seapen-reared fish where crowding and stress enhance parasite transmission. In the Bay of Fundy, fish farming has grown rapidly, from a single experimental farm operation in 1984 to well over 50 farms in the Passamaquoddy Bay – Bay of Fundy area in 1991. With this rapid rise in numbers of fish in the area, the abundance of sea lice has also increased, with at least one farm suffering significant losses due to these parasites within the last 4 yr. The most common of the sea lice in this area is *Caligus elongatus*. Preadults and adults move freely over the fish surface, browsing on mucus and epithelial cells. Nauplii hatch from the egg strings of the females and are free living. The copepodid larva that moults from the second nauplius is planktonic and infective to fish.

Under laboratory conditions, copepodid larvae swim upwards in the water column for several seconds and then rest, sinking for a similar length of time. Light is generally recognized as being the major environmental cue that controls vertical movement of zooplankton (Forward 1976). Gravity and other factors such as current often shape the response to light. This behaviour of sea lice in particular is believed to keep the infective larvae in the general vicinity of their fish host.

The objective of the present research was to determine if the copepodid larvae of *C. elongatus* do in fact respond to light. These investigations were designed to understand more about the behaviour of the copepodid larvae which may affect their success in infecting a host. The ultrastructure of the copepodid eyes was also investigated to gain some insight into the nature of light reception in this species.

Materials and Methods

Source of Material

Atlantic salmon (*Salmo salar*) were removed from seapen cages at Huntsman Marine Science Centre and were held in seawater tubs while adult female *C. elongatus* were collected from them using smooth forceps. The gravid females were kept in 2000-mL beakers in aerated seawater at ambient seawater temperature (12–14°C during September and October 1990). Within 1 wk, many hundreds of infective copepodid larvae were swimming in the water. Larvae were collected by filtering the water through acetate fabric; the larvae were shaken free from the material into fresh seawater and separated into groups of 20. Larvae were maintained in aerated seawater until used in the experiments.

Experimental Design

To test the response of copepodid larvae to white light, the following experiments were conducted. Plexiglas rectangular chambers, 50 cm long × 5 cm wide × 5 cm high, were filled with ambient previously aerated seawater and placed inside a

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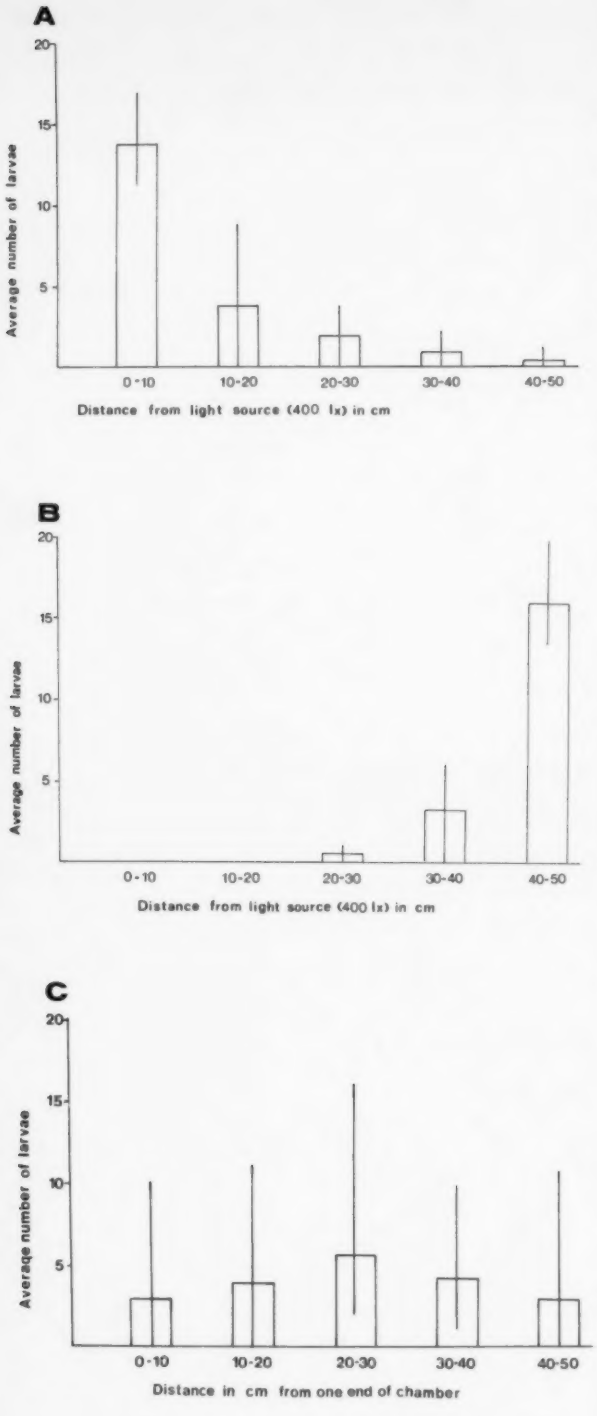

FIG. 1. Distribution of copepodid larvae of *C. elongatus* located in each 10-cm partition of a 50-cm test chamber after 5 min of exposure to a 400-lx vertically oriented white light when originally placed (A) 25 cm from the light source and (B) 50 cm from the light source and (C) after 5 min in the dark (originally placed in the middle of the chamber).

container of running seawater to maintain the temperature. In experiments to test larval response to light, a white light of 400 lx was shone vertically down at one end of the chamber into the water through an opening 5 × 5 cm. The rest of the chamber was covered to block out other light. Control experiments were conducted in the same chambers but the chambers were completely shielded from the light. Twenty copepodid larvae were added either to the centre of the chamber (in both lighted and control experiments) or to the end furthest from the light (in light experiments only). The experiments ran for 5 min at which time the cover was removed and dividers fitted into the chamber at 10-cm intervals to stop any further migration of the larvae. The larvae in each division of the chamber were counted under a dissecting microscope. All experiments were repeated 20 times. The average number of larvae in each chamber was compared with the number in the chamber next to it using a Mann–Whitney test to determine if there were significantly more larvae in chambers nearer the light than away from the light (in the experiments using light) and to determine whether numbers of larvae were significantly different in any one chamber in experiments conducted in the dark.

Structure of the Eyes

Copepodid larvae were examined and photographed live under a coverslip using a Leitz Ortholux microscope. Other specimens were fixed in Karnovky's fixative, pH 7.2, for 24 h, rinsed in three changes of sodium cacodylate buffer, and post-fixed in 1% osmium tetroxide for 2 h. Specimens were dehydrated in acetone and embedded in Epon 812/araldite. Thick (1.0 μm) and thin (60 nm) sections were cut horizontally, longitudinally, and transversely through the eyes and were stained with Toluidine blue (thick sections) or uranyl acetate and lead citrate (thin sections). Light micrographs were taken from the thick sections and ultramicrographs from the thin sections using a Philips EM 400T electron microscope.

Results

Response to Light

The copepodid larvae of *C. elongatus* are positively attracted to vertically directed 400-lx white light from a distance of 25 cm but not from a distance of 50 cm. An average of 14 of 20 larvae, when placed 25 cm from the light source, travelled to the area immediately below the light source (Fig. 1A). Decreasing numbers of larvae were found progressively further from the light source. Mann–Whitney analysis showed significant differences ($p < 0.05$) in the number of larvae found in each partition of the experimental chamber. Larvae placed 50 cm from the light source appeared to stay in that area, with the number of larvae in the partition furthest from the light being significantly greater than the number of larvae elsewhere (Fig. 1B). These larvae were probably not stationary, however, since random movement in three of four directions would have brought them up against a wall, thus detaining them. Larvae placed in the chamber and left in the dark moved randomly, with no significant differences in numbers among the divisions of the chambers (Fig. 1C).

Structure of the Eyes

The copepodid larvae of *C. elongatus* has paired eyes located dorsally underneath a transparent carapace midway anteriopos-

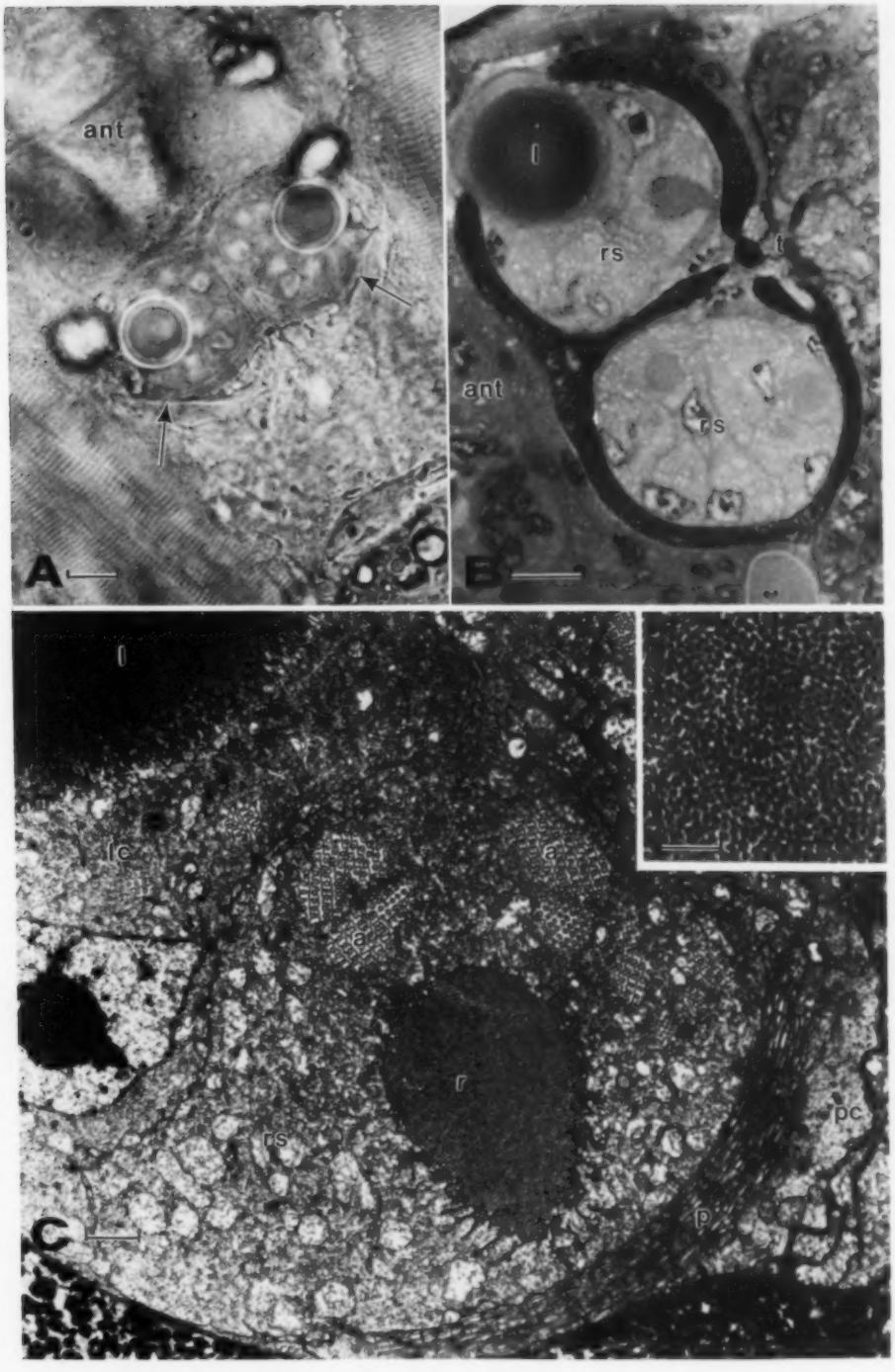

FIG. 2. (A) Light micrograph of the paired eyes (arrows) of *C. elongatus*. ant, anterior. Bar = 25 μm. (B) Light micrograph of a 1.0-μm horizontal section through the paired eyes. The depth of section through each eye is not identical; thus the lens of only one eye is visible. l, lens; rs, rhabdomeric sensory cells, t, triangular sensory area located below and between the two eyes. Bar = 12 μm. (C) Electron micrograph showing an overview of the eye. lc, lens cell; r, rhabdomere; a, amplifier; p, platelets; pc, platelet cell. Bar = 2.0 μm. Inset: high magnification of the lens. Bar = 0.2 μm.

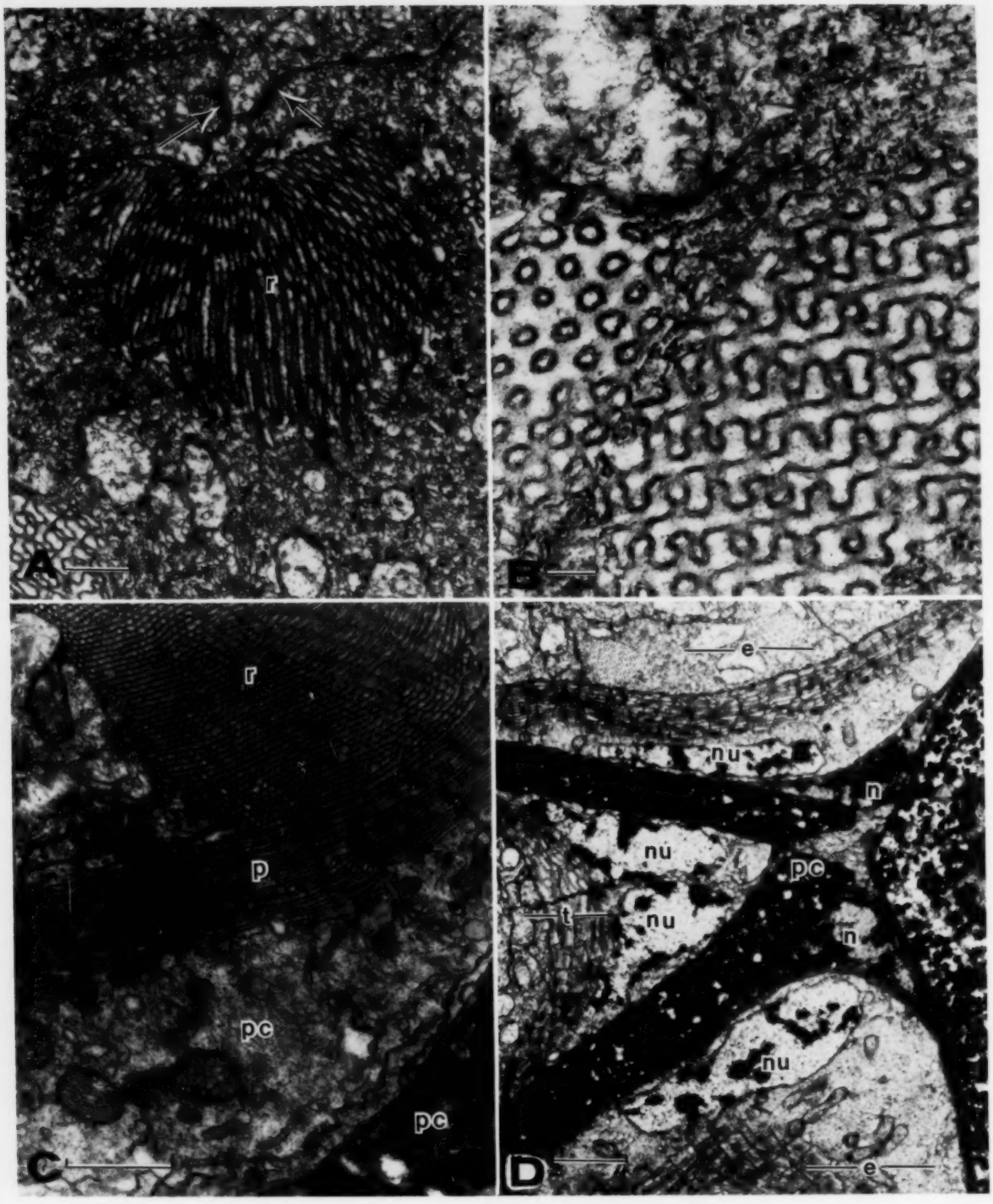

FIG. 3. (A) Electron micrograph of the rhabdomere (r) showing its formation from the plasma membrane (arrows) of the sensory cell. Bar = 0.5 μm. (B) Electron micrograph of the double membrane tubes and scrolls located within the sensory cells. Bar = 0.25 μm. (C) Electron micrograph of a platelet cell (upper pc) showing the platelets (p). r, rhabdomere of sensory cell; lower pc, pigment cup cell. Bar = 1.0 μm. (D) Electron micrograph showing the abutment of the two eyes (e) and the triangular sensory area (t). pc, pigment cup; n, nucleus of pigment cup cell; nu, nuclei of platelet cells. Bar = 2.0 μm.

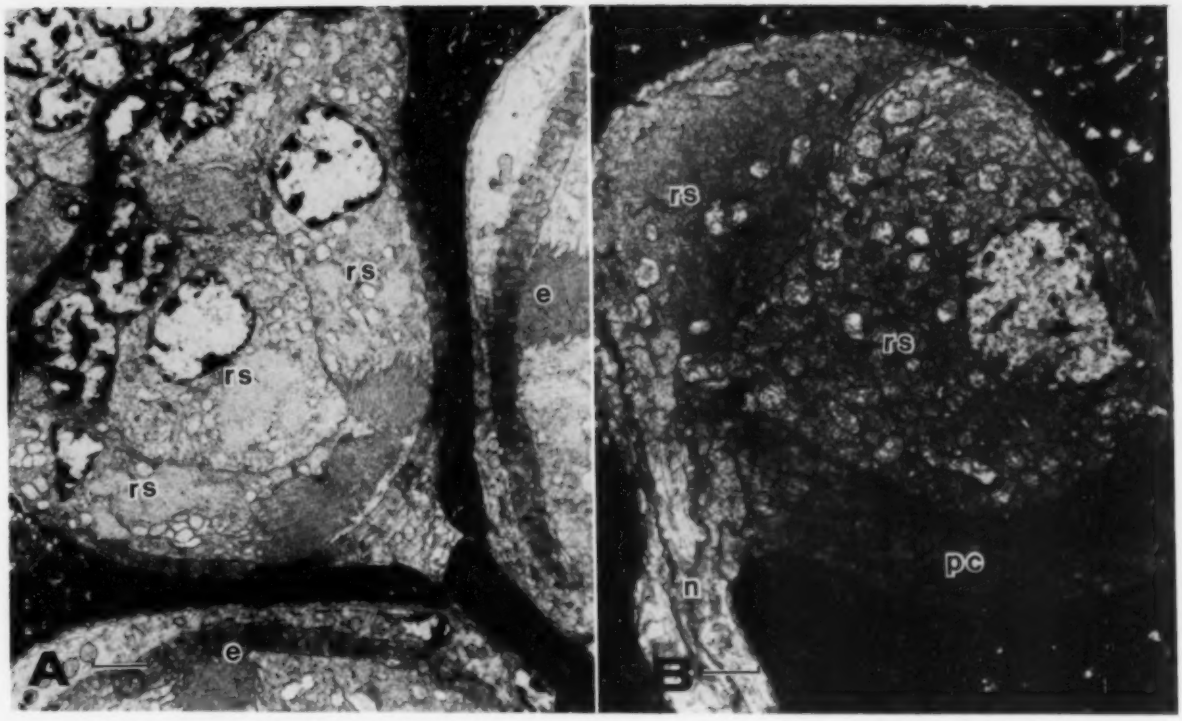

FIG. 4. (A) Electron micrograph of the triangular sensory area located between and below the two eyes (e). rs, rhabdomeric sensory cells. Bar = 2.0 μm. (B) Electron micrograph showing the exit of the nerve axons (n) of the rhabdomeric sensory cells and the platelet cells (pc) through the pigment cup cell. Bar = 1.0 μm.

teriorly down the length of the cephalothorax. Each eye has a lens directed anteriolaterally located in the dorsal part of the eye (Fig. 2A). Thick (1.0-μm) sections show each eye to be contained by a pigment granule cup, which does not extend over the lens. The pigment cup of each eye has at least one gap posterioventrally which allows contact of the sensory cells of each eye with a small triangular lensless third eye located between and below the two main eyes (Fig. 2B).

Ultrastructural examination of the eyes indicates that the pigment granules of the cup lie within a nucleated cell. Overlying the pigment cup is a thin connective tissue layer that stretches over the entire eye. Within the pigment cup are the lens and lens cell, the six rhabdomeric sensory cells, and the platelet cells that bracket the base of all of the rhabdomeric sensory cells (Fig. 2C). The lens has a crystalline appearance (Fig. 2C, inset) and is contained by the lens cell which is nucleated. The lens cell sometimes contains rhabdomeres and usually contains regularly arranged membranous coils and scrolls, here referred to as amplifiers (see below and Discussion), mitochondria, and some glycogen.

The rhabdomeric sensory cells run dorsoventrally the full length of the eye. They are nucleated and contain the rhabdomeres, which are long microvillilike internal amplifications of the outer plasma membrane (Fig. 3A). The rhabdomeres arise from the plasma membrane of the outer lateral surface of the sensory cells and extend ventrally to come into close contact with the platelets of the platelet cell below. The sensory cells also contain mitochondria, glycogen, and an extensive convoluted smooth endoplasmic reticulum. These cells contain numerous large areas containing regular arrangements of tubes, scrolls, or coils of double membranes, here called amplifiers (Fig. 3B). These membranes run at various angles to the path of light entering the eye.

The platelet cells occupy the ventral and lateral part of the eye lying outside the sensory cells and inside the pigment cup cell. The inner aspect of each platelet cell contains layers of membranes, similar to the rhabdomeres, but running horizontally. The platelet cells together form a three-dimensional bracket around the rhabdomeric cells (Fig. 3C). The exact number of platelet cells is difficult to determine.

A triangular area lying between and below the two eyes also contains at least two rhabdomeric sensory cells and at least two platelet cells, with the platelet cells bracketing the sensory cells anteriorly (Fig. 3D and 4A). This triangular eye is not always enclosed posteriorly by pigment cup granules. The rhabdomeric sensory cells and platelet cells are unipolar, with the nerve axons of each cell exiting either one of the two main eyes through gaps in the pigment cup posterioventrally, either via the triangular eye or sometimes lateral to the triangular eye (Fig. 4B). A composite three-dimensional interpretation of one of the two main eyes is shown in Fig. 5.

Discussion

Copepodid Response to Light

The experimental design of the chamber and the lighting arrangement to test the copepodid response to light was not intended to mimic the physical environment of the copepods in nature. The chamber itself was designed horizontally to equalize

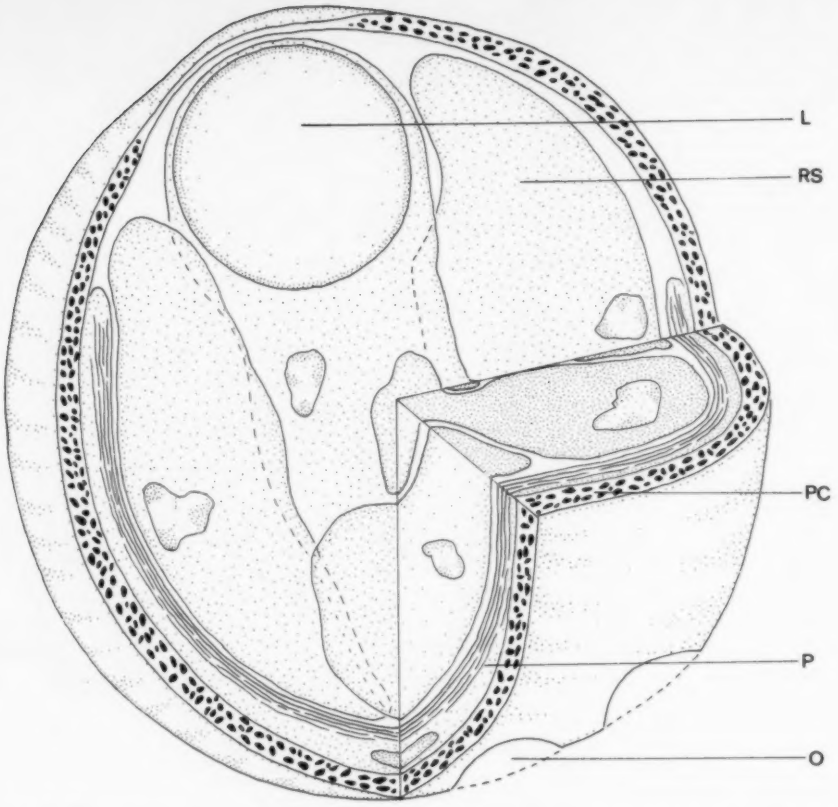

FIG. 5. Composite reconstruction of one of the eyes of *C. elongatus* (not to scale). L, lens; RS rhabdomeric sensory cell; PC, pigment cell cup; P, platelets in platelet cell; O, opening that allows contact with triangular eye.

the influence of gravity, and the larvae were thus responding horizontally when under natural conditions they would swim vertically. Likewise, a bright white light was used and copepods in nature are exposed to differing wavelengths of light depending on their depth in the water. The experimental design was such that the light was bright at one end of the chamber but decreased rapidly in intensity and was dim at the centre of the chamber where the larvae were introduced. Lythgoe (1988) discussed extensively various aspects to consider when experimenting with light and vision in water; any one experimental design, however, cannot encompass all possible parameters that may affect vision under natural conditions. Using the present experimental conditions, the larvae of *C. elongatus* responded positively to light at a distance of 25 cm but not at a distance of 50 cm. When placed at a distance of 50 cm, most larvae were still concentrated in that area after 5 min. It is likely that these larvae moved randomly, but movement in three of four directions would bring them against a wall which would then turn them back into the original chamber. They did not move in any one particular direction when kept entirely in the dark, somewhat more of the larvae being located near the area into which they were introduced. Most zooplankton show either positive or negative responses to light (Forward 1976). The knowledge that sea lice do move towards lighted areas of the water is useful to the aquaculture industry. For example, further experiments could test whether the shading of seapen cages or the sinking of cages into darker waters would reduce the abundance of lice on fish.

Structure of the Eyes

Copepods have evolved several eye designs (Land 1983). The eyes of *C. elongatus* are of the small lens type not uncommon amongst the Copepoda (Cronin 1988). The eyes described here are similar at the histological level to eyes described from the copepodid stage of *Caligus curtis* by Heegard (1947) and from *Caligus diaphanus* and *Lepeophtheirus nordmannii* by Vaissière (1961). The complete ultrastructure of caligid eyes is described here for the first time. This ultrastructure suggests that these eyes use the lens–mirror type of optics, which Land (1983) suggested was common in copepod nauplii. Heegard (1947) demonstrated that the naupliar eyes of *C. curtis* are developmental stages of the same eyes present in the copepodid stage. Thus the lens–mirror type of eye may be the common type of eye for copepod larval stages.

The crystalline lens converges incoming light which is then detected by the six rhabdomeric sensory cells. Sensory pigment is reported by Land (1983) to be associated with the rhabdomeres, which are common structures found in light-sensitive cells. The double membraneous tubes, scrolls, and coils

arranged in differing directions to the path of light are unusual and have not been reported before from crustaceans. They increase the membrane surface area and may therefore increase area available for sensory pigment, but the fact that they run in different directions suggests that they are not channeling or focussing information. They may maximize absorption of light but not direct it, and they are thus referred to here as amplifiers. The structure of these membranes is reminiscent of ciliary receptors in the eyes of some platyhelminths as shown by Fournier (1984) in her review.

The platelet cells are positioned so as to receive information from the rhabdomeres and may be the concave, reflecting "mirror" cells described from other crustaceans at the light microscope level by Land (1983). Land (1983) suggested that these may redirect the light back through the sensory cells and thus increase the "photon catch." The numerous membrane lamellae in these cells, however, suggest that they also may be photosensitive. Cronin (1988) suggested that small lens eyes could enhance directional and absolute sensitivity to light.

The small triangular eye contains structures believed to be photosensitive. Its position and the orientation of the sensory cells and platelet cells suggest that it may pick up light impinging on the copepod ventrally.

It would seem that the copepodid larvae of *C. elongatus* has eyes well adapted to detect even very low intensities of light from all directions. The eyes may sense some directionality of the light source and may be able to optimize light detection using membraneous organelles referred to here as amplifiers. It is likely that light stimulates swimming, and as Swift and Forward (1988) have suggested for larvae of the insect *Chaoborus puntipennis*, gravity may orient the larvae to swim upwards under natural conditions. The present experiments do show, however, that the copepodid larvae actually orient to the light. These larvae are not predatious while free swimming, and thus their response to light may be designed to keep them in the water column in the vicinity of their fish hosts.

Acknowledgements

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Community Structure of Lacustrine Macrobenthos: Do Taxon-based and Size-based Approaches Yield Similar Insights?

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Rodríguez, M.A., and P. Magnan. 1993. Community structure of lacustrine macrobenthos: do taxon-based and size-based approaches yield similar insights? Can. J. Fish. Aquat. Sci. 50: 800–815.

We sampled the macrobenthos of three Laurentian Shield lakes to evaluate how the taxonomic structure and the size distribution (biomass spectra) of littoral communities varied (1) seasonally over the summer, (2) among lakes, and (3) in relation to substratum. Seasonal variation was limited: although total numbers differed significantly among months, the taxonomic structure (biomass) and the biomass size spectrum for each lake–year combination were relatively constant during the summer. Taxonomic structure differed significantly among lake–year combinations; substratum variables explained 55% of this variation. Biomass spectra were conspicuously bimodal for all lake–date combinations, with a main peak at 6.4–102.4 mg dry mass and a lesser peak at 0.1–0.4 mg dry mass. These spectra differ considerably from those reported previously for marine and lacustrine macrobenthos. The shape of the biomass spectrum was relatively invariant across months and lakes, which is remarkable in view of the differences in taxonomic structure, substratum, and fish species composition among lakes. This conservative pattern provides indirect evidence for structuring of littoral macrobenthic communities by size-dependent processes. The results illustrate that taxon-based and size-based approaches can emphasize strikingly different aspects of community structure.

Nous avons échantillonné le macrobenthos de trois lacs du bouclier laurentien pour évaluer comment la structure taxonomique et la distribution en taille (biomasse) des communautés littorales variaient 1) mensuellement au cours de l'été, 2) entre les lacs et 3) en relation avec le substrat. La variation saisonnière était limitée : bien que les nombres totaux différaient significativement entre les mois, la structure taxonomique (biomasse) et la distribution en taille de chaque combinaison lac–année étaient relativement constantes pendant l'été. La structure taxonomique était significativement différente entre les combinaisons lac–année; les variables associées au substrat expliquaient 55 % de cette variation. La structure en taille était manifestement bimodale pour toutes les combinaisons lac-date avec un pic principal à 6,4–102,4 mg poids sec et un moindre à 0,1–0,4 mg poids sec. Ce patron diffère considérablement de ceux rapportés précédemment pour le macrobenthos marin et lacustre. La forme de la distribution en taille était relativement invariable entre les mois et les lacs, ce qui est remarquable si on considère les différences qui existaient entre les lacs dans la structure taxonomique, le substrat et la composition des espèces de poissons. Ce patron conservateur fournit l'évidence indirecte que le processus structurant les communautés macrobenthiques littorales est dépendant de la taille. Les résultats illustrent que les approches basées sur le niveau taxonomique et sur la taille peuvent mettre en lumière des aspects très différents de la structure des communautés.

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Aggregated community variables such as total numbers, total biomass, and secondary production are often successfully used as descriptors of lacustrine zoobenthos in studies relating the zoobenthos to abiotic factors, primary production, or fish (Ball and Hayne 1952, Hanson and Leggett 1982; Dermott 1988; Rasmussen and Kalff 1987; Rasmussen 1988; Plante and Downing 1989). However, understanding of the mechanisms underlying biotic interactions, abiotic influences, and mass and energy transfers (internal flows, imports, and exports) generally requires more detailed characterizations of community structure.

Prevailing approaches to the evaluation of zoobenthic community structure are either taxon based or size based (Strayer 1991). Taxon-based approaches usually center on quantitative analyses of taxon abundances or densities (by numbers or mass), often relying on multivariate statistical techniques. Among the main objectives of this type of analysis are to (1) detect relationships between the taxonomic structure of a community and environmental variables, which may include pollutants, and (2) evaluate spatiotemporal variability. Recent examples are provided by Leland et al. (1986), Vincent (1986), Johnson and Wiederholm (1989), Cocito et al. (1990), McCall and Soster (1990), van der Meer (1991), and Warwick and Clarke (1991). Size-based approaches focus instead on the frequency distribution of body size, the total numbers or biomass of organisms in the community usually being sorted into logarithmic intervals of some measure of organism size, such as body mass or equivalent spherical diameter (Sheldon et al. 1972). Although this approach originally was developed in studies of energy transfer in plankton communities, more recently it has been used to evaluate spatial and temporal variation in lacustrine zoobenthos communities (Mittelbach 1981; Strayer 1986;

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TABLE 1. Composition (g DM·m^{-2}) of lake substrata. For each substratum category, the median is given along with lower and upper quartiles (in parentheses).

Lake	Vegetation	Organic detritus	Sand (0.6–2.0 mm)	Gravel (2–32 mm)
Marnac 1985	7.9 (2.3, 19.4)	795.3 (294.6, 1222.8)	6157.8 (4266.1, 9920.5)	954.2 (482.0, 1938.1)
Marnac 1986	8.0 (1.8, 15.2)	270.3 (145.6, 544.2)	8621.7 (5142.9, 11664.5)	1532.0 (746.6, 1870.6)
Sauterelle	0.3 (0.0, 3.8)	932.5 (707.9, 1315.0)	1122.1 (111.9, 3506.0)	471.4 (59.7, 1046.0)
Vautour	48.8 (28.6, 122.5)	336.1 (88.2, 1141.7)	167.5 (115.3, 312.8)	16.9 (11.4, 28.4)

Hanson et al. 1989) and to relate community size distributions to environmental features (Hanson 1990). To date, very few studies have characterized the size structure of lacustrine zoobenthos (Strayer 1991), and virtually all the information available comes from only three lakes, studied by Mittelbach (1981), Strayer (1986), Hanson et al. (1989), and Hanson (1990).

Here, we apply both taxon-based and size-based analyses to the macrobenthic communities of three Laurentian Shield lakes differing in fish species composition and substratum type to evaluate (1) seasonal variation, (2) variation among lakes, and (3) influence of substratum. The same sampling and processing methods were used for the three lakes, one of which was sampled on two consecutive years. To our knowledge, taxon-based and size-based analyses of zoobenthos have not previously been combined in a single study, and thus we compare the two approaches and discuss the salient aspects of community structure revealed by each.

Materials and Methods

Description of Lakes

We studied Lake Marnac in the St-Maurice Reserve (47°05′N, 73°15′W) and Lakes Sauterelle and Vautour in the Mastigouche Reserve (46°38′N, 73°15′W) in Quebec, Canada. The three lakes are small (9.7, 7.5, and 7.3 ha for Marnac, Sauterelle, and Vautour, respectively), shallow (maximum depth: 16.8, 13.7, and 16.8 m; mean depth: 8.6, 5.3, and 4.8 m), and thermally stratified in the summer. During the summers of 1985 and 1986, Secchi transparency ranged from 2.1 to 5.0 m, and oxygen concentration in the epilimnion ranged from 8.7 to 12.6 mg·L^{-1}. Microhabitats in the littoral zone were identified by SCUBA diving (cf. Laughlin and Werner 1980; Eadie and Keast 1981; Mittelbach 1981). Lakes Marnac and Sauterelle have a single littoral microhabitat characterized by a mixture of sand, gravel, and organic detritus (Table 1). Lake Vautour has two distinct microhabitats in the littoral zone, one of sand and gravel covered with scattered stems of *Sparganium* spp. and the other of sand, gravel, and organic detritus (Table 1); these two microhabitats cover approximately equal areas. Fish species composition differs among lakes: six species (brook trout (*Salvelinus fontinalis*), northern redbelly dace (*Phoxinus eos*), white sucker (*Catostomus commersoni*), pearl dace (*Margariscus margarita*, formerly *Semotilus margarita*), creek chub (*Semotilus atromaculatus*), and common shiner (*Luxilus cornutus*, formerly *Notropis cornutus*)) are present in Lake Marnac, four species (brook trout, northern redbelly dace, white sucker, and pearl dace) are present in Lake Sauterelle, and two species (brook trout and northern redbelly dace) are present in Lake Vautour.

Sampling of Macrobenthos and Bottom Substrata

Samples were collected from the littoral (<2 m) and profundal (>4 m) zones of the three lakes on 24–30 May, 18–22 June, 11–17 July, and 7–12 August 1985 and from Lake Marnac on 20–22 May, 16–18 June, 16–17 July, and 11–12 August 1986. Three stations were arbitrarily sampled from each littoral microhabitat (sand – gravel – organic detritus for Lakes Marnac and Sauterelle; *Sparganium*-sand-gravel and sand – gravel – organic detritus for Lake Vautour), and one station was sampled from the profundal zone, near the center of each lake. All samples were collected between 08:00 and 18:00 with a standard Ekman dredge (231 cm^2). On each sampling date, three samples were collected at each littoral station, yielding nine littoral samples for Lakes Marnac and Sauterelle and 18 littoral samples for Lake Vautour. Due to logistical problems, only 10 littoral samples were collected from Lake Vautour in May 1985. Three samples were collected at profundal stations, except in Lake Marnac, August 1985 (one), Lake Sauterelle, May 1985 (two) and August 1985 (one), and Lake Vautour, June 1985 (two) and July 1985 (two). In the field, samples were sifted through a No. 30 US (0.6-mm mesh) sieve and the retained fraction was preserved in a 25% formalin solution.

In the laboratory, macrobenthic organisms were generally identified to family, counted, and then transferred to 95% ethanol. For each taxon, length–weight relationships were estimated from a subsample of 50 organisms (Mittelbach 1981). Individual organisms were measured (total length and width of the cephalic capsule) to the nearest 0.01 mm and weighed to the nearest 0.01 mg after drying for 24 h at 80°C. Biomass estimates for all samples were expressed as dry mass (DM) per unit area (milligrams per square metre).

The substratum was sorted into two organic categories (''live vegetation'' and ''organic detritus'') and two inorganic categories (''sand'' and ''gravel''). Organic and inorganic components were separated by flotation in a saturated sucrose solution, and the live vegetation (mainly green parts) was picked from the supernatant material. Sand was separated from gravel by sifting through a sieve (2-mm mesh). Pebbles and rocks (particles retained by a 32-mm mesh) were extremely rare in our samples and were excluded from subsequent analyses. After sorting, substratum components were dried for 24 h at 80°C and weighed to the nearest 0.01 g. The composition of lake substrata (Table 1) was estimated by averaging the abundance of each substratum component (grams per square metre DM) across stations and across months.

Because benthos variables measured over small spatial scales are likely to show some degree of spatial autocorrelation, samples collected at a station may not be statistically independent (Thrush 1991). Results for the three samples from each station were thus pooled to obtain composite macrobenthos samples and composite substratum samples that more closely met the requirement of independence.

Quantitative Analyses

Total abundance

Analysis of variance (ANOVA) was used to examine the effects of seasonal change and among-lake variation on total numerical density (number of organisms per square metre) and total biomass (milligrams per square metre) of littoral macrobenthos. To avoid confounding among-lake variation with year-to-year variation, we examined the 1985 and 1986 data separately. Data for 1985 were analyzed with factorial ANOVA with month (four categories) and lake (three categories) as main factors. Data for Lake Marnac in 1986 were analyzed with one-way ANOVA.

Multivariate analyses based on taxonomic structure

Following graphical examination of the data, the transformation $\ln(X + 1)$ was applied to the taxon abundances and to the substratum variables to stabilize the variances and to more closely approximate normal distributions. Differences between lake–year combinations (Marnac 1985, Marnac 1986, Sauterelle 1985, and Vautour 1985) and between zones (littoral and profundal) were coded as binary dummy variables assuming values of 0 or 1 (e.g., samples from the littoral zone of Lake Marnac in 1985 had a value of 1 for the dummy variable "marnac85," a value of 0 for all other dummy variables representing lake–year combinations, and a value of 0 for the dummy variable coding for zone). Chironomidae larvae and pupae, both of which were abundant in our samples, were treated as separate taxa in the multivariate analyses because chironomids at these two developmental stages differ in their ecology and behavior (Hilsenhoff 1991).

We used canonical correspondence analysis (ter Braak 1986, 1987), a nonlinear eigenvector ordination method that directly relates community attributes (typically relative abundances) to environmental variables, for evaluation of community–environment relationships. In situations where responses of taxa to environmental gradients are unimodal rather than linear, canonical correspondence analysis can perform better than conventional linear techniques for relating two data matrices, such as canonical correlation analysis and redundancy analysis (ter Braak 1987). Canonical correspondence analysis (CANOCO program, version 3.10; ter Braak 1991) was used to relate variation in taxonomic structure (relative abundances, based on biomass, of the 16 most abundant macrobenthic taxa) to (1) differences between lake zones (littoral versus profundal), (2) differences among lake–year combinations (Marnac 1985, Marnac 1986, Sauterelle 1985, and Vautour 1985), (3) seasonal change, and (4) differences in substratum characteristics.

Because littoral community structure was significantly influenced by among-lake variation and by substratum characteristics (see Results), the method of Borcard et al. (1992) was slightly modified to partition the total variation in taxonomic structure of littoral macrobenthos into four components, each of which could be assigned to an independent source of variation: (1) within-lake variation related to substratum characteristic, (2) among-lake variation related to substratum characteristics, (3) among-lake variation related to factors other than substratum, and (4) unexplained (residual) variation. These components were estimated as outlined below.

Lake–year dummy variables are invariant for all samples from a given lake and year and thus reflect variation among lake–year combinations, but provide no information on within-lake variation. In contrast, substratum variables are free to vary from sample to sample and therefore provide information on both within-lake variation and variation among lake–year combinations. The variation explained jointly by the lake–year dummy variables and the substratum variables is the total explained variation; the remaining portion is the unexplained (residual) variation. The total explained variation was broken down into three independent components: (1) within-lake variation related to substatum characteristics (estimated as the variation explained by substratum variables after statistical removal, by partial canonical correspondence analysis, of the influence of lake–year variables), (2) among-lake variation related to substratum characteristics (estimated as the total variation explained by substratum variables minus quantity (1) above, or, alternatively, by the total variation explained by lake-year variables minus quantity (3) below), and (3) among-lake variation related to factors other than substratum (estimated as the variation explained by lake–year variables after statistical removal, by partial canonical correspondence analysis, of the influence of sustratum variables).

Variables that contributed most to the explanation of relative abundances of taxa were chosen by the forward selection procedure in CANOCO, with a 5% significance level as a cutoff point. Variance inflation factors in final models were always less than 6.0, indicating that collinearity among variables was relatively low. Significance tests for final models relating community composition to spatiotemporal and substratum variables were based on Monte Carlo permutation tests for the sum of all eigenvalues, as implemented in CANOCO. The significance of relationships between individual taxa and environmental variables was evaluated by approximate *t*-tests for the canonical coefficients (ter Braak 1990).

To check for robustness of results to choice of statistical method, two linear techniques were used in addition to canonical correspondence analysis. Discriminant function analysis (SYSTAT program; Wilkinson 1988) was used to determine (1) whether samples could be accurately assigned to lake-year combinations on the basis of taxa abundances (biomass) and (2) which taxa discriminated best among lake–year combinations. Canonical correlation analysis (SYSTAT program; Wikinson 1988) was used to relate variation in taxonomic structure (log-transformed biomass of the 16 most abundant macrobenthic taxa) to differences in substratum characteristics. Because of the differences between canonical correspondence analysis (nonlinearity, taxon abundances standardized by sample total) and the other two techniques (linearity, taxon abundances not standardized), agreement between canonical correspondence analysis and the linear techniques is likely to arise from real patterns in the data and not from methodological artifacts.

Characterization of biomass spectra

Biomass size distributions were analyzed only for the littoral zone because sample sizes and macrobenthos abundance in the profundal zone were insufficient for adequate characterization of biomass spectra. We established 13 biomass size classes that covered the entire range of individual body mass in our samples (0.01–85.81 mg DM). Class boundaries were on a $\log_2$ scale, with class 1 encompassing organisms with body mass <0.025 mg DM and class 13 encompassing organisms with body mass between 51.2 and 102.4 mg DM. For each lake and date, biomass size spectra (Sheldon et al. 1972) were generated by summing for each size class the mass of all organisms falling within the size class, regardless of taxonomic affiliation.

Normalized size spectra (see Kerr 1972, Sheldon et al. 1977,

TABLE 2. Summary statistics for canonical correspondence analysis of macrobenthos relative abundance in relation to (a) dummy variables coding for zone and lake–year, (b) dummy variables coding for lake–year, and (c) substratum variables (*$p < 0.05$).

	Correlations with ordination axes			Canonical coefficients		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
			(a) Zone and lake–year effects			
Zone	0.88*	0.08	−0.03	0.96*	0.30*	−0.24
Marnac 1985	0.08	−0.54*	−0.32*	−0.03	−0.39*	−1.18*
Marnac 1986	0.25*	−0.14	0.53*	0.00	0.00	0.00
Sauterelle	0.02	−0.11	−0.05	−0.05	0.05	−0.77*
Vautour	−0.29*	0.65*	−0.15	−0.15*	−0.79*	−1.08*
			(b) Lake–year effect			
Marnac 1985	−0.61*	0.27*	0.33*	−0.35*	1.16*	0.47*
Marnac 1986	−0.23	−0.60*	−0.04	0.00	0.00	0.00
Sauterelle	−0.13	0.20	−0.59*	0.09	1.05*	−0.72*
Vautour	0.77*	0.09	0.21	0.81*	1.10*	0.25
			(c) Substratum effect			
Organic detritus	−0.20	0.15	−0.53*	−0.03	0.05	−0.13*
Vegetation	0.50*	0.11	0.20	0.13*	0.02	−0.00
Sand	−0.60*	−0.33*	0.11	0.07	−0.47*	−0.08
Gravel	−0.68*	−0.05	0.17	−0.31*	0.41*	0.11

and Silvert and Platt 1978 for theoretical treatment of normalized size spectra) were generated by calculating for each size class the expression $\log_2$ (total biomass in size class/size class range). Least squares linear regressions of this expression on $\log_2$ (upper boundary of size class) were used to quantify differences in biomass size spectra among lakes and to interpret the distribution of biomass among size-classes. Regression statistics (e.g., the slope and the dispersion about the regression line) of the normalized size spectra have been used before to quantify and compare size distributions of pelagic plankton (lakes versus ocean: Sprules et al. 1983; Sprules and Munawar 1986) and of lake benthos (seasonal change and zonal variation: Hanson et al. 1989; habitat-related variation: Hanson 1990).

Results

Total Abundance

Macrobenthos abundance (numbers and biomass) and number of taxa were higher in the littoral zone than in the profundal zone (Appendix 1). Many taxa (Caenidae, Ceratopogonidae, Chironomidae larvae and pupae, Gammaridae, Gomphidae, Ephemeridae, Libellulidae, Polycentropidae, and Tabanidae) were common in the littoral zone whereas Chaoboridae larvae and Chironomidae larvae were dominant, and other taxa uncommon, in the profundal zone (Appendix 1). For the 1985 data, ANOVA indicates that total numerical densities in the littoral zone varied significantly among lakes ($p < 0.001$) and throughout the summer ($p < 0.001$); the lake by month interaction was not significant ($p = 0.17$). Total littoral biomass varied significantly among lakes ($p = 0.003$), but the month effect ($p = 0.12$) and the lake by month interaction ($p = 0.66$) were not significant. Similar results were obtained with the 1986 data for Lake Marnac, which indicate an effect of month on total numerical density ($p = 0.04$) and no effect of month on total biomass ($p = 0.24$). Month-to-month variation in littoral abundances did not follow a linear temporal trend: abundances were generally lowest in July (Appendix 1).

Taxonomic Structure

In a canonical correspondence analysis of macrobenthos with lake–year, zone, and month as predictors, zone (littoral versus profundal) was the first variable to enter in the forward selection procedure, followed by lake–year variables (Table 2a). Month was not included in the final model, indicating the absence of a seasonal trend in relative abundances. The final model accounts for 33.4% of the total variation in the data set and has a permutational probability for the sum of all eigenvalues of $p = 0.001$ after 1000 permutations. The first ordination axis (24.1% of total variation) is associated with major differences in relative abundances between the littoral zone and the profundal zone, as indicated by the high correlation ($r = 0.88$) between axis scores and the dummy variable coding for zone (Table 2a). The first axis reflects primarily a contrast in the abundance of Chaoboridae larvae, which were absent from the littoral zone but were very abundant in the profundal zone (Appendix 1).

When ecological sample sets are disjunct, having subsets of related samples that bear little taxonomic resemblance to each other, it is best to separate the samples into subsets suitable for further analysis (Green 1980). In view of the marked differences in taxonomic structure between the littoral and profundal zones, all subsequent analyses focused on littoral macrobenthos (sample sizes for the profundal zone were too small for separate multivariate analyses).

Canonical correspondence analysis of littoral macrobenthos with lake–year and month as independent variables yielded significant differences in community composition among lake–year combinations and an absence of seasonal trend (month did not enter in the forward selection procedure; Table 2b), match-

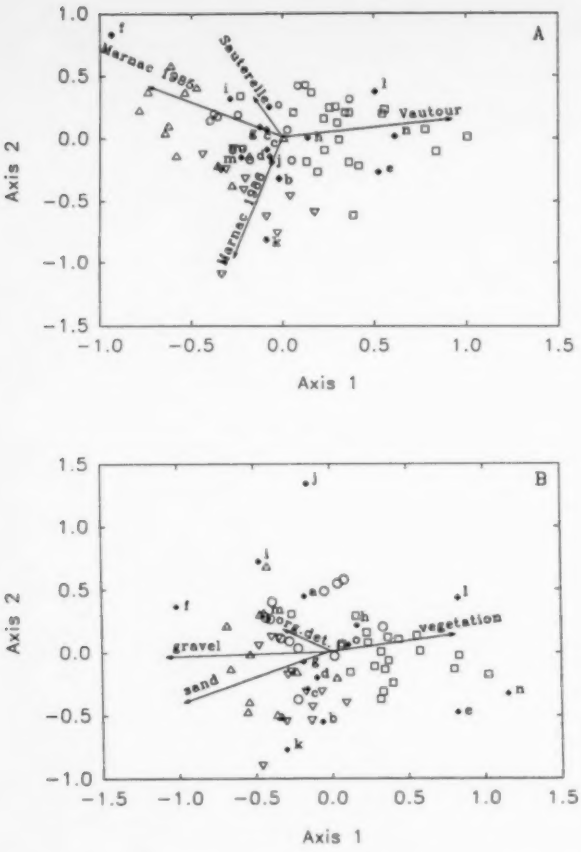


FIG. 1. Canonical correspondence analysis ordinations of littoral macrobenthos. (A) Ordination of samples and macrobenthic taxa in relation to dummy variables coding for lake–year combinations; (B) ordination of samples and macrobenthic taxa in relation to substratum variables. The length of the projection of a variable's arrow onto an ordination axis is proportional to the correlation between the variable and the axis. Arrow origins coincide with the centroid of sample points. Triangles: Lake Marnac 1985; inverted triangles: Lake Marnac 1986; open circles: Lake Sauterelle; open squares: Lake Vautour; solid diamonds represent individual taxa: a, Caenidae; b, Ceratopogonidae; c, Chironomidae larvae; d, Chironomidae pupae; e, Coenagrionidae; f, Elmidae; g, Ephemeridae; h, Gammaridae; i, Gomphidae; j, Hydroptilidae; k, Leptoblephidae; l, Libellulidae; m, Polycentropidae; n, Sialidae; o, Tabanidae.

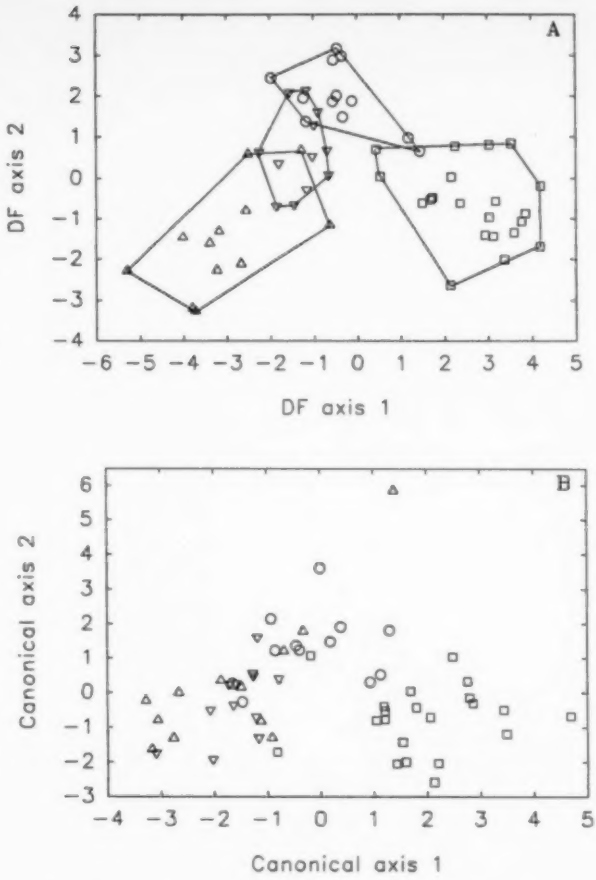


FIG. 2. (A) Ordination of littoral samples along the first two discriminant function axes; (B) Ordination of littoral samples along the first two canonical correlation axes. Convex polygons enclose the samples from each lake. Triangles: Lake Marnac 1985; inverted triangles: Lake Marnac 1986; open circles: Lake Sauterelle; open squares: Lake Vautour.

ing the results obtained earlier for littoral samples (total biomass). The final model accounts for 21% of the total variation in littoral samples and has a permutational $p = 0.001$ after 1000 permutations. Lakes Marnac 1985 and Vautour have extreme values along the first axis (12.8% of total variation) whereas Lakes Marnac 1986 and Sauterelle have intermediate values along this axis. The second axis (4.4% of total variation) separates Lake Marnac 1986 from the three other lake–year combinations (Fig. 1A; Table 2b). Axis 3 (3.8% of total variation) separates Lake Sauterelle from the three other lake–year combinations (Table 2b). Among-lake differences in 1985 are generally comparable with the difference between Lake Marnac in 1985 and 1986 (Fig. 1A; Table 2b). The taxa contributing most to the discrimination among lake–year combinations (Fig. 1A; approximate t-tests for the canonical coefficients with $p < 0.05$) are Elmidae (high relative abundance in Lake Marnac 1985), Leptoblephidae (high relative abundance in Lake Marnac 1986), Coenagrionidae, Libellulidae, and Sialidae (high relative abundances in Lake Vautour).

Canonical correspondence analysis of littoral macrobenthos with substratum variables as predictors revealed a significant relationship between community composition and substratum features (Fig. 1B; Table 2c). The final model accounts for 19.6% of the total variation in littoral samples and has a permutational $p = 0.001$ after 1000 permutations. Axis 1 (10.7% of total variation) reflects primarily a gradient in inorganic components (sand and gravel) versus vegetation whereas axis 2 (4.9% of total variation) is most strongly related to sand (Fig. 1B; Table 2c). The taxa contributing most to separation of lakes along the inorganic component versus vegetation gradient are Libellulidae, Sialidae, Coenagrionidae, and Elmidae (Fig. 1B).

TABLE 3. Summary statistics for discriminant function analysis of macrobenthos relative abundance in relation to dummy variables coding for lake–year. The multivariate significance values for axes 1 and 2 are $p < 0.001$; the significance value for axis 3 is $p = 0.09$.

Classification accuracy = 86%

	Predicted				
Observed	Marnac 1985	Marnac 1986	Sauterelle	Vautour	Total
Marnac 1985	9	3	0	0	12
Marnac 1986	0	9	3	0	12
Sauterelle	0	0	12	0	12
Vautour	0	1	1	19	21
Total	9	13	16	19	57

Contribution of individual taxa to discrimination among lake–year combinations

	Correlations with ordination axes (canonical loadings)		Canonical coefficients		
Taxon	Axis 1	Axis 2	Axis 1	Axis 2	Univariate p (ANOVA)[a]
Caenidae	0.01	0.13	0.08	0.39	0.529
Ceratopogonidae	0.07	−0.26	0.61	0.01	0.001
Chironomidae larvae	0.03	−0.35	−0.37	−0.42	0.004
Chironomidae pupae	0.03	−0.20	−0.20	−0.44	0.119
Coenagrionidae	0.22	−0.16	0.37	0.03	0.001
Elmidae	−0.29	−0.49	−0.57	−0.90	<0.001
Ephemeridae	−0.03	−0.18	0.24	−0.12	0.274
Gammaridae	0.35	0.10	0.62	0.08	<0.001
Gomphidae	−0.08	0.08	0.21	0.34	0.168
Hydroptilidae	0.01	−0.00	−0.25	0.42	0.979
Leptoblephidae	−0.01	0.17	−0.07	0.46	0.295
Libellulidae	0.47	−0.21	0.79	−0.32	<0.001
Polycentropidae	−0.05	−0.04	−0.19	0.28	0.835
Sialidae	0.27	−0.16	0.57	−0.31	<0.001
Tabanidae	0.01	−0.19	0.03	−0.23	0.336

[a]Probability level corresponding to one-way ANOVA of log-transformed taxon biomass with the four lake–year combinations as treatments.

Sample positions and taxon positions in the ordination based on substratum features (Fig. 1B) are broadly comparable with those in the ordination based on lake–year combinations (Fig. 1A). This suggests that for the littoral zone the marked differences in substratum among lake–year combinations (Table 1) explain a substantial portion of the variation in community composition among lake–year combinations.

A breakdown of the total variation showed that lake–year variables and substratum variables together account for 29.0% of the total variation in community composition, leaving 71.0% unexplained. Within-lake variation related to substratum characteristics accounts for 8.0% of the total variation, or 27.6% of the explained variation. Among-lake variation related to substratum variables accounts for 11.6% of the total variation, or 40.0% of the explained variation. Among-lake variation related to factors other than substratum accounts for 9.4% of the total variation, or 32.4% of the explained variation. Among-lake variation related to substratum variables accounts for 55.2% (11.6/(11.6 + 9.4)) of the among-lake variation; this fraction is relatively large, as suggested above on the basis of the similarity between the ordinations in Fig. 1A and 1B.

Discriminant function analysis of littoral macrobenthos with lake–year as independent variables (Fig. 2A; Table 3) yields results generally comparable with those obtained by canonical correspondence analysis (Fig. 1A; Table 2b). Lake–year combinations are clearly differentiated on the basis of taxa abundances (biomass) with a classification accuracy of 86%. The multivariate diagnostics and the univariate statistics for individual taxa (Table 3) indicate that Libellulidae, Gammaridae, Elmidae, and Sialidae contribute most to discrimination; nevertheless, several other taxa also differ significantly in biomass among lake–year combinations (Table 3; cf. Appendix 1).

Canonical correlation analysis results also generally agree with those of canonical correspondence analysis: first, taxonomic structure is significantly related to substratum characteristics, in particular to a gradient in vegetation versus inorganic components (Fig. 2B; Table 4); second, variation in taxonomic structure among lakes reflects differences in substratum among lakes (Fig. 2B). The taxa contributing most to separation of lakes along the inorganic component versus vegetation gradient are Libellulidae, Gammaridae, Sialidae, and Coenagrionidae (Table 4). Because of the different taxon response models assumed by canonical correlation analysis and canonical correspondence analysis, there is some disagreement between the two techniques with respect to the relative contribution of particular taxa to the ordination axes and to the response of taxa to substratum variables. This is most notable for Gammaridae, which are linearly related (bivariate linear regressions of log-

TABLE 4. Summary statistics for canonical correlation analysis of macrobenthos relative abundance in relation to substratum variables. Overall multivariate significance (Wilk's lambda) is $p < 0.001$. The canonical correlations for the first three axes are 0.87, 0.76, and 0.65. The multivariate significance values for axes 1 and 2 are $p < 0.001$; the significance value for axis 3 is $p = 0.01$. The total redundancy (percentage of variation in taxa abundances explained by the four substratum variables) is 17.3%.

Intraset correlations and canonical coefficients for substratum variables

	Correlations with ordination axes		Canonical coefficients	
Variable	Axis 1	Axis 2	Axis 1	Axis 2
Vegetation	0.45	0.11	0.54	0.18
Organic detritus	0.01	−0.04	0.16	0.08
Sand	−0.87	0.03	−0.92	1.93
Gravel	−0.75	−0.43	0.06	−2.14

Intraset correlations and canonical coefficients for individual taxa

	Correlations with ordination axes (canonical loadings)		Canonical coefficients		
Taxon	Axis 1	Axis 2	Axis 1	Axis 2	Multiple regression p^a
Caenidae	0.11	0.22	0.31	0.26	0.093
Ceratopogonidae	0.02	−0.41	0.24	−0.59	0.004
Chironomidae larvae	0.02	−0.27	−0.39	−0.07	0.016
Chironomidae pupae	0.09	−0.08	0.15	−0.14	0.383
Coenagrionidae	0.20	−0.20	−0.08	−0.02	0.007
Elmidae	−0.11	0.25	−0.06	0.09	0.019
Ephemeridae	0.05	0.06	0.22	0.22	0.568
Gammaridae	0.53	0.26	0.51	0.25	<0.001
Gomphidae	0.02	0.39	0.17	0.39	0.040
Hydroptilidae	0.15	0.38	0.04	0.66	0.005
Leptoblephidae	0.05	0.11	0.09	0.11	0.202
Libellulidae	0.61	−0.15	0.74	−0.17	<0.001
Polycentropidae	0.00	0.11	−0.08	0.19	0.507
Sialidae	0.31	−0.27	0.60	−0.36	<0.001
Tabanidae	0.10	−0.04	0.27	−0.30	0.362

[a]Probability level corresponding to a multiple regression of log-transformed taxon biomass on the four substratum variables.

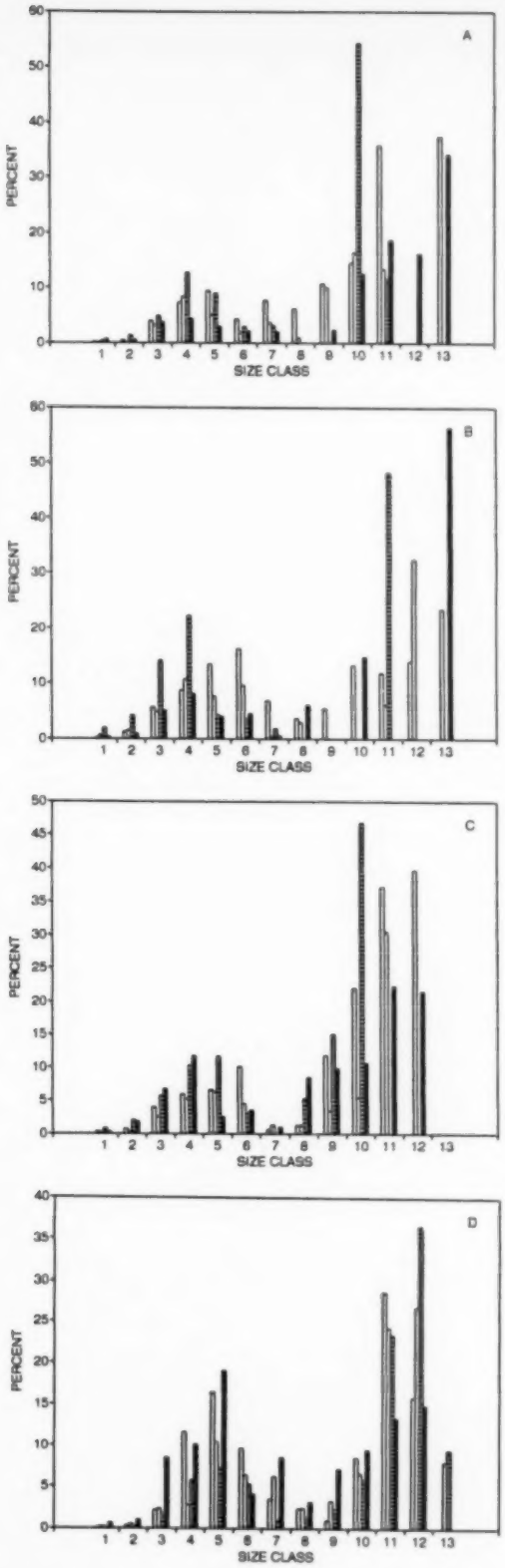
TABLE 5. Summary of regressions for normalized biomass spectra (based on summer averages).

Lake–year	Intercept	SE of intercept	Slope	SE of slope	MSE	r^2	p	n
Marnac 1985	6.20	0.41	−0.60	0.11	1.44	0.74	<0.001	13
Marnac 1986	6.34	0.38	−0.72	0.10	1.36	0.82	<0.001	13
Sauterelle	5.64	0.42	−0.56	0.12	1.47	0.67	0.001	12
Vautour	6.50	0.49	−0.64	0.13	1.75	0.69	<0.001	13
All lakes combined	6.17	0.21	−0.63	0.06	1.47	0.72	<0.001	51

transformed mass on log-transformed substratum variables) to sand ($r = -0.55$; $p < 0.001$), gravel ($r = -0.40$; $p = 0.002$), and vegetation ($r = 0.55$; $p < 0.001$) and thus contribute strongly in the linear analysis (Table 4) but not in the nonlinear analysis (Fig. 1B). Second-order polynomials were also fitted to test for possible nonlinearity, but the quadratic term in these models was never significant.

Biomass Spectra

Size-based analyses were performed only for littoral samples (see Materials and Methods). The biomass spectra of all lake–date combinations were conspicuously bimodal, with a main peak in size classes 10–13 (6.4–102.4 mg DM), a lesser peak centered on size classes 4–5 (0.1–0.4 mg DM), and a trough centered on size classes 7–8 (0.8–3.2 mg DM) (Fig. 3). There is a remarkable constancy in the overall shape of the size distribution across months and lakes, as evidenced by the normalized biomass spectra (Fig. 4). Normalized biomass spectra calculated for each lake from averages over the four summer months clearly illustrate the general resemblance among lakes (Fig. 5). Analysis of covariance of the normalized biomass spectra calculated from summer averages indicated that the slopes ($p = 0.79$) and intercepts ($p = 0.53$) do not significantly differ among the four lake–year combinations (significance values are approximate because residuals are autocorrelated). Therefore, summer averages for the lake–year



combinations were pooled to estimate a common regression line (Table 5; Fig. 5).

Regression slopes near -1 with little dispersion about the regression line can be expected whenever the size distribution is relatively homogeneous across size classes, i.e., when the biomass spectrum is flat (Kerr 1974; Sheldon et al. 1977). The regression diagnostics (Table 5) show that the slope estimates for each of the lake–year combinations and for the pooled data are significantly different from -1 and that the magnitude of the differences is relatively large. These differences reflect an overabundance of large bodied organisms (≈ 1.0 mg and larger) relative to smaller-bodied organisms (Fig. 3). Considerable dispersion exists about the regression lines, as indicated by the r^2 and MSE values (Table 5). This results mainly from the bimodality of the biomass spectra as evidenced by the pattern of deviations from the common regression line (residuals), which are highest for size classes corresponding to the two major peaks in the biomass spectra (Fig. 5).

Each of the peaks can be associated with particular taxa (Appendix 2). Diptera (mostly Chironomidae larvae, followed by Ceratopogonidae and Chironomidae pupae) and Amphipoda (Gammaridae) contribute most to the smaller peak on the left of the biomass spectra (Fig. 3). The larger peak to the right of the biomass spectra (Fig. 3) is formed mainly by Ephemeroptera (Ephemeridae) and Odonata (Gomphidae, Libellulidae, and Macromiidae) (Appendix 2).

To determine whether size class distributions for littoral samples ($n = 57$) were significantly related to substratum features, we used the 13 size classes as dependent variables in a canonical correspondence analysis with substratum variables as predictors. No significant association was found between size class variables and substratum variables. In a subsequent analysis, size classes 1–7, which correspond approximately to the smaller peak in the biomass spectra, were aggregated into one group, and size classes 8–13, which correspond to the larger peak, were aggregated into another. These two groups were then used as dependent variables in a canonical correspondence analysis with substratum variables as predictors. As in the previous analysis, no significant association was found between the two sets of variables. We also attempted to relate the size class distribution to substratum characteristics by multiple linear regression, with the slopes of the normalized biomass spectra for the 57 littoral samples as the dependent variable and substratum variables as predictors. All possible regressions were tested: none was significant.

Discussion

The results illustrate that taxon-based and size-based approaches can emphasize strikingly different aspects of community structure. Taxon-based analyses highlighted the differences among lake–year combinations and related these differences to variation in substratum characteristics. In comparison, size-based analyses underscored the similarity between lake–date combinations and did not link the size distribution to substratum features.

FIG. 3. Biomass spectra for the four lake–year combinations. (A) Lake Marnac 1985; (B) Lake Marnac 1986; (C) Lake Sauterelle; (D) Lake Vautour. First open bars: May; second open bars: June; striped bars: July, solid bars: August.

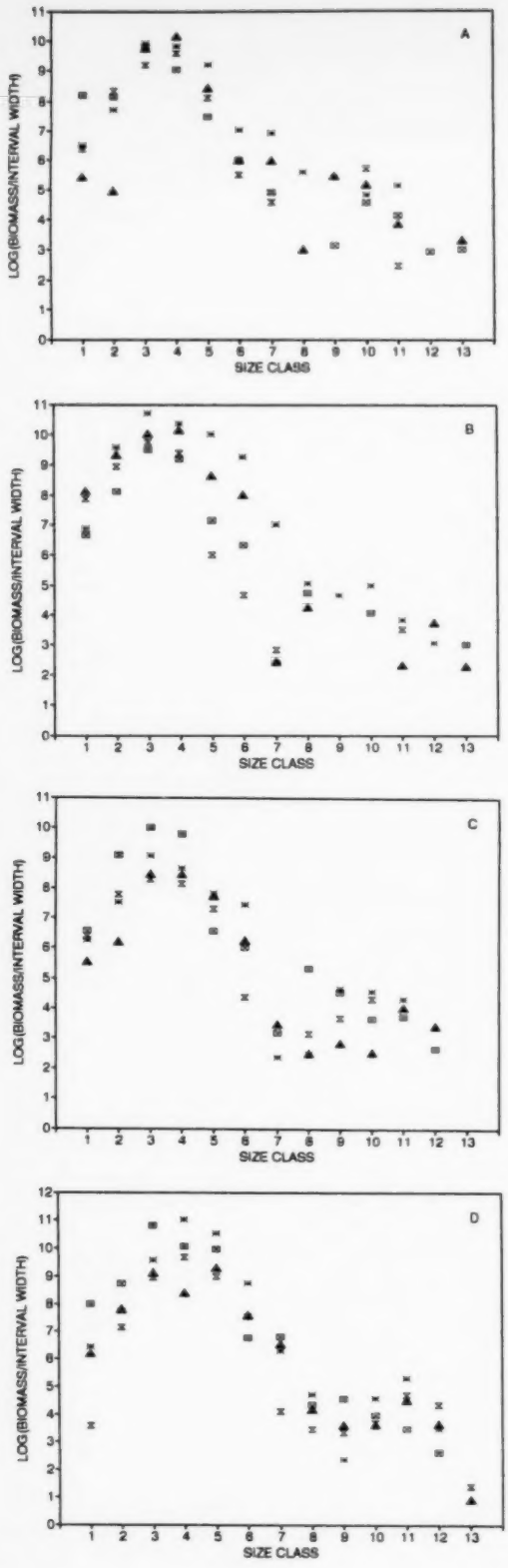


FIG. 4. Normalized biomass spectra for the four lake–year combinations. (A) Lake Marnac 1985; (B) Lake Marnac 1986; (C) Lake Sauterelle; (D) Lake Vautour. Asterisks: May; solid triangles: June; hourglass symbols: July; crossed squares: August.

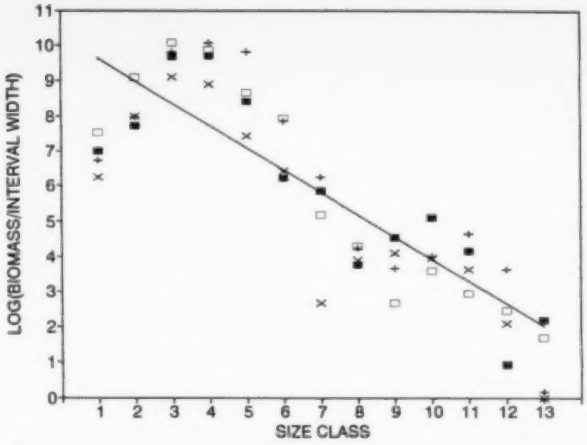


FIG. 5. Seasonally averaged normalized size spectra. The regression line is for data pooled across lake–year combinations. Solid squares: Lake Marnac 1985; open squares: Lake Marnac 1986; crosses: Lake Sauterelle; plus signs: Lake Vautour.

The strong contrast in taxonomic structure between the taxa- and organism-rich littoral communities and the relatively depauperate profundal communities is not surprising given the marked differences in oxygen availability, temperature, sedimentation regime, primary productivity, and habitat structure between the two zones (Jónasson 1978). Because sampling did not cover the entire profundal zone, our conclusions strictly apply only to the profundal zone near the center of the lake. Although only one profundal station was sampled from each lake, consistent differences between the littoral samples and the profundal samples were found in all lake–year combinations.

For littoral communities, lake–year combinations differed substantially in taxonomic structure, and among-lake variation related to substratum variables explained 55% of this difference; the remaining 45% must be accounted for otherwise (e.g., differential fish predation, substratum effects not measured by our substratum variables, random differences).

Seasonal variation in littoral communities was limited: although total numbers differed significantly among months, the taxonomic structure based on biomass and the overall shape of the biomass spectrum for each lake–year combination were relatively constant during the summer. In contrast, previous studies of littoral macrobenthos in temperate lakes have reported marked variation in size distributions during the summer. Mittelbach (1981) found that larger organisms declined across the summer in a small marl lake in Michigan and concluded that this pattern could be due to selective predation by fish, emergence of late instars and the appearance of new generations, or both. Hanson et al. (1989) found that littoral, sublittoral, and profundal biomass spectra varied considerably during the summer in a small lake in Alberta and attributed this variation to a variety of factors: losses by emergence of adult insects, post-breeding mortality of amphipods, mollusks, and leeches, indi-

vidual growth, and the appearance of small size classes due to reproduction. Biomass spectra for two distinct types of weedbed in the littoral zone, *Chara* beds and rooted-plant weedbeds, also were seasonally variable when the two weedbed types were considered separately (Hanson 1990).

The bimodal biomass spectra in the present study also differ considerably in shape from the size distributions reported previously for marine and lacustrine benthos (reviewed in Strayer 1991). Schwinghamer's (1981) study of marine benthos shows a strongly unimodal biomass spectrum over the biomass range covered in the present study (10^{-2}–10^{2} mg DM). In lakes, the shape of size distributions for littoral benthos is usually quite variable both among lakes and within lakes. Unimodal size distributions were found by Mittelbach (1981) (log-normal distribution of body lengths) and by Strayer (1986) (a minimum near 10^{-6} mg DM and a plateau in the range 10^{-2}–10^{0} mg DM; data were lacking for larger organisms). Biomass spectra were highly variable during summer in the study of Hanson et al. (1989), but the summer average for littoral benthos was bimodal in the range 10^{-1}–10^{2} mg DM (assuming dry mass = 0.15× wet mass), with a major peak at 1.2–2.4 mg DM and a minor peak at 38–76 mg DM (in our study, there was a minor peak at 0.1–0.4 mg DM and a major peak at 6.4–102.4 mg DM). In the same lake, summer-averaged biomass spectra were essentially flat for *Chara* beds and were weakly bimodal for rooted plant weedbeds, which had a strong peak at 0.6–1.2 mg DM and a much weaker peak at 4.8–9.6 mg DM (Hanson 1990). In contrast with our study, analysis of biomass spectra regression slopes demonstrated a strong relationship between environmental features (weedbed type) and biomass spectra.

The choice of mesh size used in sieving benthos samples can influence the apparent shape of the biomass spectrum and the seasonal patterns of variation, in particular for the lower end of the spectrum. Early instars of *Chironomus* are lost when 0.3-mm sieves are used instead of 0.1-mm sieves (Shiozawa and Barnes 1977). Mittelbach (1981) used a 0.18-mm sieve in his study and found that a large fraction of the prey collected were less than 1.0 mm in length. Most organisms this small would have passed through the 0.6-mm sieve used in our study. Our results could thus underestimate the abundance and seasonal variability of the smallest size classes. If so, the lower peak of the apparent biomass spectrum would be somewhat shifted to the right relative to the "true" spectrum, and additional peaks at the left end of the spectrum could have passed undetected. Presumably, mesh-related biases would mainly affect the results for size class 1 (<0.025 mg DM) because organisms small enough to pass through the 0.6-mm sieve are likely to fall within the lowest biomass class. In our samples the mean lengths of organisms in the lower biomass classes are 2.75 mm for size class 1, 2.92 mm for size class 2, 3.30 mm for size class 3, 4.22 mm for size class 4, and 5.43 mm for size class 5.

Differences in sampling techniques could also influence comparisons with other published studies. In Schwinghamer's (1981) study, macrofauna were sieved through a 0.42-mm mesh, meiofauna were collected on a 0.01-mm mesh, and bacteria up to 0.01 mm minimum dimension were counted from whole sediment samples. Strayer (1986) counted all visible metazoans from unpreserved core samples. Sediment samples were sieved through a 0.5-mm screen in the studies of Hanson et al. (1989) and Hanson (1990); the authors of the former study felt that organisms less than ≈0.015 mg DM were not adequately sampled. Differences among studies in the overall shape, seasonal variability, and relationship to substratum of the biomass spectrum cannot be explained by differences in sampling techniques because, as discussed above, studies using a relatively coarse (0.5–0.6 mm) mesh size would underestimate the abundance and seasonal variability only for the left end of the spectrum.

The relative invariance of biomass spectra in the study lakes is remarkable in view of the differences in taxonomic structure, substratum, and fish species composition (hence, presumably fish predation) among lake–year combinations. This conservative pattern provides indirect evidence for structuring of littoral macrobenthic communities by size-dependent processes. At least six possible mechanisms contributing to the creation and maintenance of bimodality can be identified. (1) Intermediate-sized organisms (0.8–3.2 mg DM, corresponding to the trough of the biomass spectrum) are consumed selectively by larger benthic predators. This certainly seems feasible given the high biomass of Odonata nymphs, which are macrophagous predators (Cummins 1973; Pennak 1978), among the larger size classes. Odonata nymphs consume mostly other insects and often are top predators in the benthos (Hilsenhoff 1991). (2) Size-related trade-offs in the feeding efficiency of nonpredatory benthos lead to a bimodal size structure, as proposed by Strayer (1991), based on the model of Lodge et al. (1988). Smaller animals should have a higher assimilation efficiency because they can selectively ingest high-quality food (algae and bacteria) without having to consume large quantities of the surrounding sediment, which is of poor nutritional quality. Larger animals cannot select food on such a fine scale and must consume bulk sediment together with algae and bacteria; nevertheless, they can compensate for the low nutritional value of the food mass by having longer guts and gut-residence times than smaller animals. Strayer (1991) suggested that selection for either a long gut (larger body size) or a selective feeding apparatus (smaller body size) may lead to a bimodal size distribution. In the present study, mayflies (Ephemeridae) dominated the larger peak of the size distribution. Mayfly nymphs are generally microphagous, feeding on organic detritus, algae, and higher aquatic plants (Cummins 1973; Pennak 1978; Hilsenhoff 1991). The smaller peak was dominated by chironomids, which usually are microphagous and consume mainly algae and organic detritus (Cummins 1973; Pennak 1978; Pinder 1986). Chironomids presumably can select their food on a finer scale than mayfly nymphs (e.g., by use of specialized mouthparts and net building). (3) Competition for food (based on mechanisms other than assimilation efficiency) or for space induces segregation into two size classes corresponding to the two peaks in the size distribution, to reduce overlap in resource use at intermediate body sizes. (4) Intermediate-sized organisms (0.8–3.2 mg DM, corresponding to the trough of the biomass spectrum) are consumed selectively by fish. This seems unlikely because lakes differing in fish species composition had very similar biomass spectra. In Lakes Sauterelle and Vautour the consumption of macrobenthic taxa by fish is highly variable during the summer and also differs considerably between the two lakes (Tremblay and Magnan 1991), yet the biomass spectra for these lakes were comparable and relatively invariant throughout the summer. (5) Bimodality of the size distribution reflects a size-dependent relationship between macrobenthic organisms and the structure of the substratum. Schwinghamer (1981) suggested that the size distribution of macrobenthos reflected the size structure of the sediments because the utilization of sediments should depend on the size of the sediment particles relative to the size of the organisms, but Warwick's

(1984) study of marine zoobenthos did not support this idea. In the present study, although taxonomic structure was shown to depend on substratum characteristics (about half of the among-lake variation in taxonomic structure was explained by substratum differences), there was no relationship between biomass spectra and substratum features. (6) Bimodality is caused by the absence of intermediate-sized taxa from a lake. This explanation does not hold for the study lakes because several abundant taxa (Chironomidae larvae, Ephemeridae) were represented at intermediate sizes, and some taxa (Sialidae, Polycentropidae) had biomass peaks at these sizes.

At present, not enough data sets on the size distributions of lacustrine benthos are available for proper evaluation of what constitutes a "common" or typical biomass spectrum (and whether a typical spectrum indeed exists), what is the scope for natural variation in biomass spectra, and what biotic and abiotic agents influence the shape of biomass spectra (Hanson 1990). Our study indicates that biomass spectra can differ little among lakes within a region. Nevertheless, the results extend still further the large scope of variability in biomass spectra already observed among other lakes. This suggests that relatively general patterns interpretable in terms of energy flow, such as those described for plankton size spectra (Sheldon et al. 1972; Sprules et al. 1983; Sprules and Munawar 1986), may be elusive in lacustrine zoobenthos.

No theory exists to predict how zoobenthic biomass spectra should respond to environmental change (e.g., variation in abiotic conditions or fish predation). This contrasts with the state of knowledge for zooplankton communities, where a key process underlying size shifts and the elimination of large-bodied species, predation, has been identified and is relatively well understood. In zooplankton the outcome of predator–prey interactions is more predictable than in zoobenthos, and the roles of predator features (body size, gape size, sensory receptors), of prey features (body size, visibility, defensive structures, migration, escape reactions), and of abiotic features (light regime, hypolimnetic refugia) (Kerfoot 1980; Zaret 1980; Kerfoot and Sih 1987) are better understood. As discussed by Strayer (1991), use of the substratum by zoobenthos may complicate the interpretation of body size relationships relative to zooplankton: first, because the substratum provides cover, fish do not select benthic prey solely by body size and are strongly influenced by prey activity, prey exposure, and prey density (see references in Strayer 1991; also Macchiusi and Baker 1991); second, benthos–substratum relationships may be size dependent; and third, freedom from the requirements of buoyancy and swimming probably allows for development within the benthos of a broader array of morphological, chemical, and behavioral defenses against predation.

The portion of total variation in taxonomic structure that was explained by the substratum variables was significant but low, about 20%, both for linear and nonlinear analyses. This may account in part for the failure of the size-based approach to detect substratum-related differences because substratum effects appear to be moderate in the littoral. It also indicates that taxon-based analyses may be generally more sensitive to environmental effects than size-based analyses, even when a coarse taxonomic resolution is used. Depending on the approach used, littoral macrobenthic communities from different lake–year combinations were perceived as being either very dissimilar or remarkably alike. The apparent complementarity of the two approaches indicates that their simultaneous use may be desirable in future studies.

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Appendices

APPENDIX 1. Means for numerical density (org·m^{-2}) and biomass (mg DM·m^{-2}) of dominant macrobenthic taxa (standard deviations are given in parentheses).

	May		June		July		August	
Taxon	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
Littoral				*Lake Marnac 1985*				
Caenidae	14.4 (14.4)	1.1 (1.0)	62.5 (58.3)	11.4 (10.2)	14.4 (14.4)	9.0 (8.0)	0.0 (0.0)	0.0 (0.0)
Ceratopogonidae	67.3 (79.5)	11.4 (14.0)	24.1 (30.0)	4.0 (6.0)	48.1 (33.3)	5.2 (3.0)	98.6 (67.0)	10.8 (9.1)
Chironomidae (larvae)	1250.6 (712.3)	228.1 (141.7)	1587.3 (324.9)	219.6 (42.8)	1447.8 (654.5)	404.9 (147.8)	1594.5 (50.5)	126.5 (17.2)
Chironomidae (pupae)	43.3 (14.4)	6.5 (4.0)	110.6 (117.5)	27.9 (39.8)	33.7 (22.0)	3.8 (1.1)	57.7 (14.4)	5.4 (2.3)
Ephemeridae	57.7 (14.4)	341.0 (232.7)	67.3 (50.7)	895.1 (725.5)	19.2 (8.3)	157.3 (171.4)	122.7 (102.3)	275.9 (252.5)
Gammaridae	591.6 (890.8)	89.0 (132.4)	77.0 (133.3)	14.0 (24.3)	72.2 (90.1)	10.4 (13.0)	12.0 (11.0)	1.2 (1.3)
Gomphidae	57.7 (52.0)	349.3 (390.9)	19.2 (16.7)	94.1 (128.5)	0.0 (0.0)	0.0 (0.0)	9.6 (16.7)	232.0 (401.8)
Leptoceridae	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.8 (8.3)	0.4 (0.8)	21.6 (21.6)	11.0 (17.6)
Libellulidae	9.6 (16.7)	13.9 (24.0)	0.0 (0.0)	0.0 (0.0)	4.8 (8.3)	5.9 (10.2)	0.0 (0.0)	0.0 (0.0)
Limnophilidae	4.8 (8.3)	82.5 (143.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Macromiidae	0.0 (0.0)	0.0 (0.0)	4.8 (8.3)	55.5 (96.2)	0.0 (0.0)	0.0 (0.0)	4.8 (8.3)	410.0 (710.1)
Polycentropidae	24.1 (30.0)	36.5 (38.1)	0.0 (0.0)	0.0 (0.0)	9.6 (8.3)	0.3 (0.3)	9.6 (8.3)	2.4 (3.0)
Tabanidae	14.4 (25.0)	52.1 (90.3)	9.6 (16.7)	38.0 (65.7)	4.8 (8.3)	5.2 (9.0)	9.6 (16.7)	127.5 (220.8)
Others[a]	202.0 (253.7)	143.1 (142.4)	43.3 (43.3)	12.6 (13.3)	28.9 (0.0)	2.5 (2.9)	19.2 (16.7)	4.2 (3.9)
Total	2313.6 (1025.1)	1236.4 (330.4)	2005.8 (300.3)	1372.3 (767.4)	1678.7 (763.9)	604.6 (321.9)	1950.5 (68.6)	1204.4 (1613.5)

APPENDIX 1. (*Continued*)

Taxon	May		June		July		August	
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
Profundal								
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Chaoboridae	259.7	116.3	173.2	57.0	14.4	2.3	0.0	0.0
(larvae)	(229.1)	(100.8)	(75.0)	(23.2)	(25.0)	(4.0)	(0.0)	(0.0)
Chironomidae	173.2	31.4	202.0	27.1	317.5	186.3	303.0	37.8
(larvae)	(229.1)	(38.4)	(132.3)	(20.5)	(442.2)	(315.6)	—	—
Chironomidae	72.2	77.9	14.4	14.9	0.0	0.0	0.0	0.0
(pupae)	(25.0)	(5.0)	(25.0)	(25.7)	(0.0)	(0.0)	(0.0)	(0.0)
Others[b]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Total	505.1	225.6	389.6	98.9	331.9	188.6	303.0	37.8
	(373.2)	(113.0)	(114.5)	(29.6)	(427.1)	(313.5)	—	—
				Lake Marnac 1986				
Littoral								
Caenidae	115.4	6.7	19.2	3.4	16.8	7.0	0.0	0.0
	(101.0)	(5.6)	(33.3)	(5.9)	(15.0)	(6.2)	(0.0)	(0.0)
Ceratopogonidae	254.9	28.7	153.9	14.2	146.7	7.2	86.6	6.3
	(101.4)	(10.4)	(8.3)	(2.2)	(127.1)	(6.9)	(100.0)	(7.5)
Chironomidae	3400.7	659.8	2332.9	296.7	1635.4	123.8	938.0	103.5
(larvae)	(1107.4)	(220.2)	(937.1)	(125.4)	(485.6)	(40.5)	(500.1)	(44.6)
Chironomidae	153.9	21.9	105.8	14.0	33.7	2.4	62.5	4.3
(pupae)	(102.4)	(14.1)	(71.2)	(5.8)	(8.3)	(1.1)	(50.7)	(3.9)
Ephemeridae	77.0	529.3	38.5	676.3	26.5	147.3	62.5	137.9
	(68.2)	(753.1)	(22.0)	(1125.2)	(34.1)	(254.9)	(65.1)	(228.8)
Gammaridae	101.0	20.1	153.9	32.0	48.1	7.9	269.4	43.9
	(87.8)	(17.4)	(133.3)	(28.7)	(44.1)	(8.4)	(245.9)	(37.0)
Gomphidae	9.6	117.7	9.6	1.3	9.6	2.4	4.8	4.2
	(16.7)	(203.8)	(16.7)	(2.2)	(8.3)	(2.7)	(8.3)	(7.2)
Leptoceridae	62.5	14.5	28.9	11.8	4.8	5.7	28.9	1.8
	(83.3)	(21.3)	(25.0)	(16.0)	(8.3)	(9.8)	(14.4)	(1.2)
Libellulidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Limnophilidae	19.2	68.1	0.0	0.0	0.0	0.0	0.0	0.0
	(33.3)	(117.9)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Macromiidae	4.8	5.5	4.8	10.3	0.0	0.0	4.8	412.7
	(8.3)	(9.5)	(8.3)	(17.9)	(0.0)	(0.0)	(8.3)	(714.9)
Polycentropidae	19.2	48.4	0.0	0.0	0.0	0.0	19.2	2.6
	(22.0)	(59.6)	(0.0)	(0.0)	(0.0)	(0.0)	(22.0)	(4.3)
Tabanidae	0.0	0.0	4.8	4.4	0.0	0.0	4.8	12.5
	(0.0)	(0.0)	(8.3)	(7.7)	(0.0)	(0.0)	(8.3)	(21.6)
Others[a]	81.8	129.9	14.4	1.9	0.0	0.0	57.7	6.0
	(50.7)	(188.0)	(14.4)	(1.8)	(0.0)	(0.0)	(43.3)	(6.3)
Total	4261.7	1534.0	2866.8	1066.4	1921.6	303.6	1520.0	733.1
	(1307.0)	(1127.5)	(1133.0)	(955.5)	(580.2)	(282.7)	(546.9)	(980.9)
Profundal								
Ceratopogonidae	0.0	0.0	86.6	12.9	43.3	6.4	14.4	1.1
	(0.0)	(0.0)	(86.6)	(13.1)	(75.0)	(11.0)	(25.0)	(1.9)
Chaoboridae	216.5	67.3	216.5	58.2	144.3	43.8	115.4	34.5
(larvae)	(156.1)	(72.7)	(156.1)	(52.4)	(108.9)	(33.7)	(108.9)	(33.1)
Chironomidae	606.1	124.2	649.4	78.8	894.7	76.9	245.3	31.0
(larvae)	(384.8)	(75.5)	(259.7)	(40.2)	(828.6)	(68.5)	(108.9)	(18.9)
Chironomidae	28.9	31.6	0.0	0.0	0.0	0.0	0.0	0.0
(pupae)	(50.0)	(54.7)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Others[b]	14.4	97.4	28.9	35.8	43.3	13.0	0.0	0.0
	(25.0)	(168.7)	(25.0)	(38.3)	(43.3)	(12.2)	(0.0)	(0.0)
Total	865.8	320.6	981.2	185.7	1125.5	140.1	375.2	66.6
	(369.9)	(181.2)	(278.3)	(32.1)	(949.4)	(89.9)	(213.5)	(47.0)

APPENDIX 1. (*Continued*)

Taxon	May		June		July		August	
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
			Lake Sauterelle 1985					
Littoral								
Caenidae	67.3	12.8	211.6	55.3	14.4	2.5	0.0	0.0
	(36.3)	(8.0)	(145.3)	(37.3)	(14.4)	(2.3)	(0.0)	(0.0)
Ceratopogonidae	9.6	2.1	14.4	2.7	28.9	3.1	3.6	0.2
	(8.3)	(2.2)	(14.4)	(2.7)	(25.0)	(3.3)	(6.2)	(0.3)
Chironomidae (larvae)	861.0	134.6	447.3	46.0	582.0	52.6	1596.9	187.5
	(371.1)	(53.8)	(180.8)	(16.2)	(186.1)	(19.0)	(739.2)	(109.9)
Chironomidae (pupae)	62.5	6.0	24.1	1.7	28.9	2.8	9.6	7.7
	(22.0)	(0.8)	(16.7)	(1.9)	(14.4)	(3.0)	(8.3)	(12.3)
Ephemeridae	19.2	215.3	28.9	409.8	19.2	117.1	14.4	86.6
	(33.3)	(373.0)	(14.4)	(235.3)	(8.3)	(55.5)	(25.0)	(150.0)
Gammaridae	168.4	24.1	115.4	21.6	163.5	29.9	422.1	49.2
	(46.4)	(8.7)	(38.2)	(12.6)	(58.3)	(16.4)	(315.0)	(35.4)
Gomphidae	33.7	209.9	0.0	0.0	4.8	18.0	27.7	186.2
	(8.3)	(138.1)	(0.0)	(0.0)	(8.3)	(31.2)	(16.3)	(189.4)
Leptoceridae	9.6	2.1	0.0	0.0	0.0	0.0	8.4	0.6
	(8.3)	(1.9)	(0.0)	(0.0)	(0.0)	(0.0)	(7.5)	(0.5)
Libellulidae	9.6	65.9	0.0	0.0	9.6	49.0	9.6	199.6
	(8.3)	(91.9)	(0.0)	(0.0)	(16.7)	(84.9)	(16.7)	(345.7)
Limnophilidae	0.0	0.0	0.0	0.0	0.0	0.0	4.8	30.1
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(8.3)	(52.1)
Macromiidae	0.0	0.0	4.8	143.2	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(8.3)	(248.1)	(0.0)	(0.0)	(0.0)	(0.0)
Polycentropidae	0.0	0.0	4.8	3.9	0.0	0.0	42.1	5.0
	(0.0)	(0.0)	(8.3)	(6.7)	(0.0)	(0.0)	(37.5)	(5.2)
Tabanidae	4.8	15.6	4.8	2.1	0.0	0.0	0.0	0.0
	(8.3)	(27.0)	(8.3)	(3.7)	(0.0)	(0.0)	(0.0)	(0.0)
Others[a]	19.2	2.3	24.1	8.8	0.0	0.0	98.6	41.5
	(22.0)	(2.2)	(30.0)	(7.8)	(0.0)	(0.0)	(73.7)	(42.2)
Total	1269.8	694.6	875.4	691.3	851.4	275.0	2190.9	759.0
	(326.8)	(419.7)	(217.1)	(288.6)	(217.9)	(92.3)	(668.5)	(575.0)
Profundal								
Ceratopogonidae	21.6	3.5	14.4	3.0	0.0	0.0	0.0	0.0
	(30.6)	(4.9)	(25.0)	(5.2)	(0.0)	(0.0)	(0.0)	(0.0)
Chaoboridae (larvae)	43.3	15.8	57.7	14.2	187.6	37.7	0.0	0.0
	(0.0)	(9.5)	(66.1)	(17.0)	(152.0)	(32.9)	(0.0)	(0.0)
Chironomidae (larvae)	0.0	0.0	533.9	49.2	230.9	19.6	216.5	22.1
	(0.0)	(0.0)	(392.8)	(34.1)	(163.9)	(16.4)	—	—
Chironomidae (pupae)	0.0	0.0	0.0	0.0	86.6	30.3	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(114.5)	(48.1)	(0.0)	(0.0)
Others[b]	0.0	0.0	14.4	1.7	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(25.0)	(3.0)	(0.0)	(0.0)	(0.0)	(0.0)
Total	64.9	19.3	620.5	68.2	505.1	87.6	216.5	22.1
	(30.6)	(14.4)	(444.3)	(45.5)	(363.1)	(72.5)	—	—
			Lake Vautour 1985					
Littoral								
Caenidae	45.7	4.9	57.7	24.0	24.1	10.0	9.0	0.4
	(35.6)	(3.7)	(84.1)	(39.9)	(52.2)	(20.9)	(17.3)	(0.6
Ceratopogonidae	110.6	30.3	52.9	8.9	73.4	12.6	57.1	13.5
	(60.1)	(14.5)	(48.0)	(9.0)	(72.5)	(17.4)	(52.2)	(15.4)
Chironomidae (larvae)	2412.2	436.5	1082.3	184.6	1298.7	215.8	2169.9	345.9
	(555.7)	(115.1)	(847.9)	(153.5)	(499.5)	(86.9)	(1000.6)	(151.8)
Chironomidae (pupae)	218.9	35.3	57.7	5.6	32.5	5.7	66.7	7.9
	(87.3)	(14.4)	(27.8)	(3.1)	(21.3)	(3.4)	(50.7)	(7.5)
Ephemeridae	15.6	398.5	21.6	435.7	31.3	677.7	18.6	50.6
	(16.3)	(371.6)	(20.5)	(278.1)	(21.2)	(384.0)	(27.0)	(72.8)
Gammaridae	885.0	201.1	249.5	87.6	120.3	31.3	1096.1	117.8
	(546.3)	(125.1)	(139.8)	(53.2)	(19.7)	(4.4)	(883.2)	(89.4)

APPENDIX 1. (*Concluded*)

Taxon	May		June		July		August	
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
Gomphidae	0.0	0.0	1.8	0.2	12.0	206.8	0.0	0.0
	(0.0)	(0.0)	(4.4)	(0.6)	(10.9)	(253.1)	(0.0)	(0.0)
Leptoceridae	14.4	2.6	21.0	10.2	0.0	0.0	9.6	0.9
	(25.0)	(4.5)	(27.2)	(16.1)	(0.0)	(0.0)	(11.8)	(1.4)
Libellulidae	61.3	412.1	31.3	143.1	33.7	226.2	60.1	174.3
	(42.5)	(356.1)	(21.8)	(77.8)	(26.9)	(245.7)	(66.1)	(115.2)
Limnophilidae	8.4	132.6	4.8	81.6	4.8	18.5	0.0	0.0
	(7.5)	(120.5)	(7.5)	(163.2)	(7.5)	(37.2)	(0.0)	(0.0)
Macromiidae	0.0	0.0	0.0	0.0	0.0	0.0	2.4	91.7
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(5.9)	(224.6)
Polycentropidae	19.2	20.1	0.0	0.0	0.0	0.0	33.7	6.2
	(22.0)	(23.8)	(0.0)	(0.0)	(0.0)	(0.0)	(58.9)	(9.7)
Tabanidae	8.4	22.3	13.8	43.3	0.0	0.0	2.4	7.8
	(7.5)	(25.1)	(13.0)	(52.3)	(0.0)	(0.0)	(5.9)	(19.2)
Others[a]	80.6	234.3	41.5	203.9	21.6	30.1	96.2	247.2
	(45.7)	(106.7)	(23.0)	(195.0)	(12.1)	(36.7)	(59.6)	(429.9)
Total	3861.2	1789.4	1636.0	1213.5	1647.4	1416.1	3588.3	1058.0
	(130.5)	(789.2)	(1096.9)	(343.8)	(591.3)	(666.4)	(944.9)	(555.4)
Profundal								
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Chaoboridae	28.9	25.0	0.0	0.0	0.0	0.0	72.2	28.1
(larvae)	(25.0)	(21.7)	(0.0)	(0.0)	(0.0)	(0.0)	(125.0)	(48.6)
Chironomidae	28.9	9.2	64.9	4.5	43.3	7.6	822.5	132.4
(larvae)	(50.0)	(16.0)	(91.8)	(6.4)	(0.0)	(5.2)	(845.0)	(144.1)
Chironomidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(pupae)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Others[b]	14.4	138.1	43.3	2.4	21.6	35.1	28.9	1.4
	(25.0)	(239.2)	(61.2)	(3.4)	(30.6)	(49.6)	(50.0)	(2.4)
Total	72.2	172.3	108.2	6.9	64.9	42.6	923.5	161.9
	(50.0)	(233.6)	(30.6)	(3.0)	(30.6)	(44.4)	(803.3)	(143.8)

[a]Aeshnidae, Baetidae, Coenagrionidae, Elmidae, Hydroptilidae, Leptoblephidae, Molannidae, Phryganeidae, Sialidae, and unidentified trichopterans.

[b]Caenidae, Chaoboridae pupae, Ephemeridae, Gammaridae, and Polycentropidae.

APPENDIX 2. Size class distribution (mg DM·m^{-2}) of individual taxa for all littoral samples combined. Values for each taxon and size class are averages calculated across lake–date combinations.

	Size class[a]													
Taxon	1 0.025	2 0.05	3 0.1	4 0.2	5 0.4	6 0.8	7 1.6	8 3.2	9 6.4	10 12.8	11 25.6	12 51.2	13 102.4	Total
Aeshnidae	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.89	4.16	0.00	11.06
Baetidae	0.00	0.00	0.00	0.09	0.00	0.00	1.15	0.00	0.00	0.00	0.00	0.00	0.00	1.24
Caenidae	0.05	0.08	0.55	1.75	2.38	3.67	0.80	0.00	0.00	0.00	0.00	0.00	0.00	9.28
Ceratopogonidae	0.09	0.32	1.68	3.29	3.02	1.48	0.18	0.00	0.00	0.00	0.00	0.00	0.00	10.07
Chironomidae larvae	2.57	6.13	32.84	60.39	55.17	42.16	17.67	3.36	0.00	15.09	0.00	0.00	0.00	235.39
Chironomidae pupae	0.06	0.38	1.16	3.08	2.40	2.39	0.46	0.00	0.00	0.00	0.00	0.00	0.00	9.93
Coenagrionidae	0.00	0.01	0.07	0.06	0.28	0.47	1.02	0.96	0.00	2.37	0.00	0.00	0.00	5.24
Elmidae	0.01	0.02	0.16	0.61	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.49
Ephemeridae	0.00	0.04	0.25	0.42	0.94	2.05	3.16	6.12	28.20	38.20	111.40	94.20	61.97	346.97
Gammaridae	0.25	0.78	4.47	12.02	23.61	6.84	0.00	0.85	0.00	0.00	0.00	0.00	0.00	48.81
Gomphidae	0.00	0.00	0.04	0.18	0.18	0.74	0.26	3.85	11.79	22.73	25.86	23.23	0.00	88.87
Hydroptilidae	0.02	0.04	0.34	0.60	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.09
Leptoceridae	0.01	0.07	0.23	0.26	0.89	0.89	0.99	0.53	0.00	0.00	0.00	0.00	0.00	3.85
Leptoblephidae	0.00	0.00	0.02	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
Libellulidae	0.00	0.00	0.04	0.04	0.62	0.80	2.58	6.27	2.10	24.15	33.82	10.19	0.00	80.62
Limnephilidae	0.00	0.00	0.00	0.03	0.00	0.12	0.19	0.67	2.73	4.40	13.45	4.25	0.00	25.84
Macromiidae	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.65	0.00	3.47	0.00	14.68	51.42	70.56
Molannidae	0.00	0.00	0.00	0.00	0.07	0.10	0.46	0.61	0.00	0.00	0.00	3.94	0.00	5.18
Phryganeidae	0.00	0.02	0.02	0.06	0.29	0.23	0.45	0.00	0.00	0.00	0.00	0.00	0.00	1.07
Polycentropidae	0.01	0.03	0.15	0.26	0.44	0.65	2.71	1.20	0.00	2.40	0.00	0.00	0.00	7.84
Sialidae	0.01	0.00	0.03	0.04	0.05	0.33	1.18	1.25	0.39	0.00	0.00	0.00	0.00	3.27
Tabanidae	0.00	0.00	0.00	0.00	0.19	0.26	1.66	0.78	2.50	11.15	4.14	0.00	0.00	20.68
Trichoptera	0.01	0.01	0.02	0.00	0.00	0.30	0.13	0.00	0.00	1.73	6.31	0.00	0.00	8.50
Total	3.09	7.94	42.07	83.18	91.41	63.48	35.39	27.10	47.71	125.69	201.87	154.65	113.39	996.97

[a]Upper limits for each size class (mg DM) are given below the size class number.

Major Ion Chemistry of Lakes on the Kenai Peninsula, Alaska

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We characterized the major ion chemistry of over 800 lakes on the Kenai Peninsula, Alaska, from a probability sample of 59 lakes (August 1988). There were two groups: alkalinity < 300 μeq/L (78% of the lakes) and alkalinity >700 μeq/L. Low-alkalinity lakes had significantly lower concentrations of base cations and silica and significantly higher average concentrations of dissolved organic carbon (DOC) than high-alkalinity lakes. Despite widespread acidic soils and bog vegetation, and resulting high DOC concentrations, none of the lakes sampled was acidic. Sulfate concentrations (~3 μeq/L) were similar in the two groups, as were Cl^- concentrations, which decreased with distance from the coast. High-alkalinity lakes were similar chemically to rivers and shallow aquifers in the region, suggesting that the high alkalinity is a product of the major weathering reactions in this terrain; the substantially different ratios of base cations in the two groups also indicate quantitative and qualitative weathering differences. Low-alkalinity lakes were at higher elevations than high-alkalinity lakes, presumably in groundwater recharge zones. Consequently, the chemical differences between the two lake groups appeared to be controlled by relatively small differences in local hydrologic setting, and possibly by differences in mineralogy along the groundwater flowpaths.

Nous avons caractérisé la composition en ions majeurs de plus de 800 lacs de la péninsule Kenai, Alaska, à partir d'une échantillonnage probabiliste de 59 lacs (août 1988). Deux groupes ont été déterminés : 1) alcalinité < 300 μéq/L (78 % des lacs) et 2) alcalinité > 700 μéq/L. Les lacs de faible alcalinité avaient des concentrations plus faibles de cations de base et de silice et des concentrations moyenne nettement plus élevées de carbone organique dissous (COD) que les lacs d'alcalinité élevée. Malgré la présence de grandes étendues de sols acides et d'une végétation typique des marécages, et la COD élevée qui en résulte, aucun des lacs échantillonnés n'était acide. La concentration de sulfate (environ 3 μéq/L) étaient semblable dans les deux groupes de lacs, tout comme la concentration de Cl^-, qui diminuait avec la distance par rapport à la côte. Les lacs ayant une forte alcalinité ressemblaient du point de vue chimique aux rivières et nappes souterraines peu profondes de la région, ce qui laisserait entendre que la forte alcalinité serait attribuable aux grandes réactions d'altération météorique qui surviennent dans ce type de terrain. Les ratios des cations de base qui diffèrent substantiellement entre les deux groupes indiquent également des différences d'altération météorique quantitatives et qualitatives. Les lacs de faible alcalinité se situaient à une altitude plus élevée que les lacs ayant une alcalinité élevée, situés probablement dans des zones d'alimentations de la nappe souterraine. Par conséquent, les différences chimiques entre les deux groupes de lacs semblent être déterminées par des différences relativement faibles dans les conditions hydrologiques locales, et peut-être par des différences du point de vue minéralogique le long des voies d'écoulement de l'eau souterraine.

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Surveys of surface waters in North America and Europe have contributed to an understanding of the nature of the resources and of impacts from air pollution. Some recent, statistically based surveys provide quantitative estimates of chemical and physical properties of populations of lakes. In the absence of abundant, high-quality, historical lake chemistry data, surveys have been used to evaluate the extent and magnitude of acidification (Baker et al. 1991a; Charles 1991). One can infer changes in lake chemistry by using spatially extensive lake chemistry data over a range of geological and hydrological lake response types and varied deposition patterns (Henriksen et al. 1988; Neary and Dillon 1988; Sullivan et al. 1988b; Forsius et al. 1990). Conclusions regarding the degree of acidification drawn largely on the basis of spatial patterns in surface water chemistry have been challenged, in part, because of the knowledge that natural processes also can cause acidification (cf. Baker et al. 1991a, 1991b; Krug and Warnick 1991). We report here on the results of a probability survey of lakes that was designed to examine some of the issues relevant to surface water acidification and lake surveys designed for other purposes.

The study area for this survey was the Kenai Peninsula located in southcentral Alaska. Alaska was selected because of the likelihood of abundant low-alkalinity waters, acidic soils, and low sulfate deposition. Based on results of surveys of lakes in the conterminous United States (Landers et al. 1988), we expected Kenai Peninsula lakes to be bicarbonate dominated, having pH >6 and alkalinity >0. This expectation was based on the observation that regions not receiving substantial acidic deposition were, with few exceptions, bicarbonate systems (Baker et al. 1990). The only anticipated exceptions would be lakes with high dissolved organic carbon (DOC) in which pH values <6 and negative alkalinity could occur. Moreover, we expected that no lakes would be acidic from deposition of marine salts (cf. Sullivan et al. 1988a). We expected concentrations of SO_4^{2-}, NO_3^-, and NH_4^+ to be low (e.g., <5 μeq/L) in the Kenai lakes, based on the presumed low deposition of S and on the high uptake rates of NO_3^- and NH_4^+ in aquatic and terrestrial systems.

Although some water chemistry data have been reported for several hundred of Alaska's lakes, most of the data are restricted to single observations of dissolved oxygen, pH, alkalinity, or

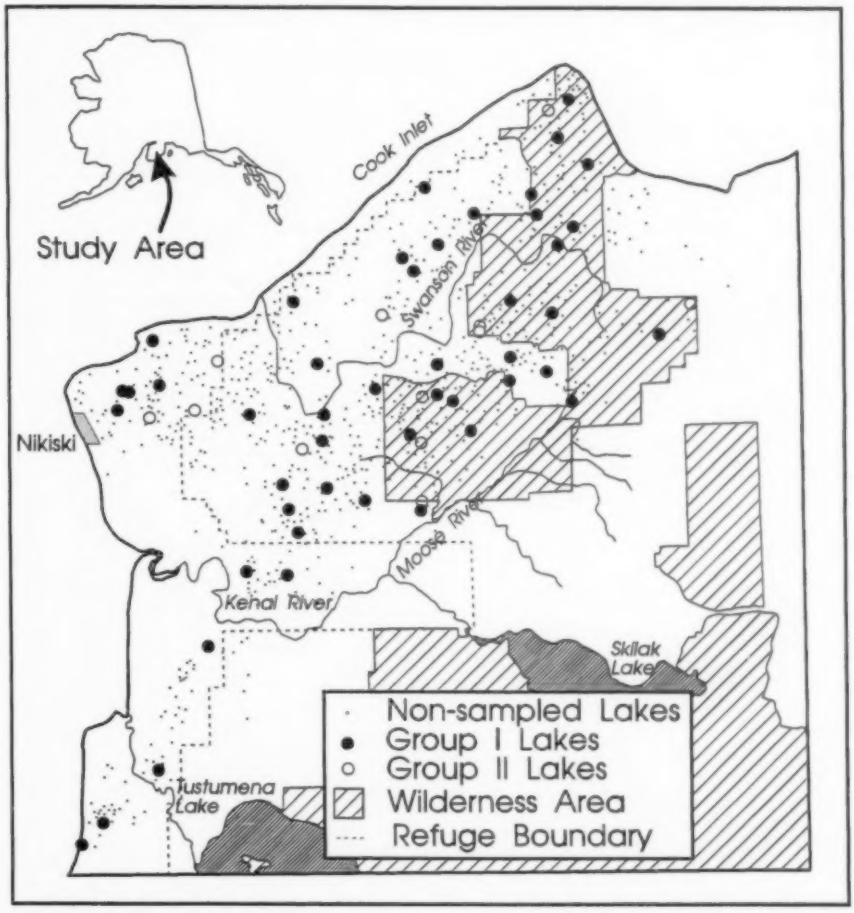

FIG. 1. Study area on the Kenai lowlands, northwestern Kenai Peninsula, Alaska. The population of lakes, as derived from 1:250000-scale U.S. Geological Survey topographic maps, is represented as small dots. Large circles identify sample lakes (n = 59): solid circles represent low-alkalinity (<300 μeq/L) lakes defined as Group I; open circles represent high-alkalinity (>700 μeq/L) lakes defined as Group II.

conductivity, with relatively few complete ion analyses (Livingstone 1966; Mauer and Woods 1987). These earlier studies were primarily for evaluation of fish habitat. This paper describes the results of a study of statistically selected lakes in the Kenai Peninsula of Alaska. The survey provides additional data for evaluating chemical distributions of low-alkalinity lakes and is the first quantitative survey of lakes in Alaska.

Study Area

At the time of the survey, limited lake chemistry data were available for selection of a study area. Most of the data in south-central Alaska were collected from lakes in three areas: the Gulkana area near Glenallen, the Nancy Lake district northwest of Anchorage, and the Kenai Peninsula south of Anchorage. Unpublished chemical data suggested that the Kenai Peninsula had the highest proportion of low-alkalinity lakes of the three areas. In addition, the Kenai Peninsula is a distinct geomorphic area, thus affording a well-defined population of lakes to sample.

The study area is within the Kenai Lowlands, a broad hummocky plain extending west of the Kenai Mountains (Fig. 1). The area is part of the Cook Inlet – Susitna Lowland within the Coastal Trough physiographic province (Wahrhaftig 1965). The topography of the Kenai Lowlands is a product of repeated Pleistocene glaciations. Karlstrom (1964) presented a detailed description of the geology and glacial history of the Kenai Lowlands subunit.

The bedrock geology of the Kenai Lowlands is sedimentary, but it is overlain by about 75–225 m of complex beds of glacial, proglacial, aeolian, and fluvial deposits (Freethey and Scully 1980). More than half the study area is composed of heterogeneous mixtures of proglacial deposits of silt, sand, and gravel interlayered with more homogeneous deposits of silt and clay (Freethey and Scully 1980). These areas usually have low relief and poor drainage, resulting in extensive areas of muskeg and bog vegetation. The second most common deposits are moraines and unsorted glacial drift, extending in an irregular band from the northeast to the southwest of the study area. They are composed of heterogeneous deposits of gravel, sand, silt, and clay with discontinuous lenses (Freethey and Scully 1980). These are generally upland areas exhibiting local relief of up to 10–15 m.

Soil samples from the southern Kenai Peninsula (Shoji et al. 1988) and from throughout southcentral Alaska, including several additional samples from the Cook Inlet area (Ping et al. 1989), indicate that most of the soils contain properties in common with both Andisols (soils developed in volcanic ash deposits) and Spodosols (organic soils characterized by leached mineral layers with precipitated Al and Fe). Virtually all of these soils show a strong influence of volcanic ash, derived from the volcanoes on the west side of the Cook Inlet. Consequently, organic carbon does not decrease regularly with depth, but instead shows two or three maxima reflecting intermittent contributions of volcanic ash (Ping et al. 1989). Where the vegetation community is composed of white spruce or tundra, organic accumulation can be sufficient to produce soils with strong spodic properties. Most of these soils are expected to have moderately low cation exchange capacities, low base saturation, and low pH (surface pH values around 4.2 to 5.0).

The northern Kenai Lowlands are drained primarily by the Swanson River to the north and the Moose River to the south, which derive most of their flow from discharge of local aquifers. Groundwater availability in the study area is highly variable, reflecting the heterogeneous deposits. There is a general trend of high groundwater yield in the Kenai–Nikiski area decreasing to low yields eastward to the Kenai Mountains (Freethey and Scully 1980). Examination of the water resources in the western portion of the study area, however, again shows the considerable heterogeneity in these general patterns, with a strong association between glacial outwash deposits and the occurrence of high-yield wells. Industrial wells in the Nikiski area are developed in artesian aquifers at about 30–90 m below the surface whereas low-yield domestic wells tap the shallow aquifers, typically <30 m in depth (Anderson and Jones 1972).

The study area is located immediately north of a climatic and vegetation transitional zone influenced by both maritime and continental climatic patterns (Watson 1959). The average annual precipitation at Kenai is 51 cm, with a range of about 30–70 cm for the period 1950–70. The mean annual temperature is 0.7°C, with average monthly temperatures ranging from about −12°C in January to 12°C in June (Anderson and Jones 1972).

Methods

The study area is the recently glaciated lowland portion of the Kenai Peninsula, including the area west of the Kenai Mountains extending across to Cook Inlet and south to Tustumena Lake. We identified 902 lakes on 1:250000-scale U.S. Geological Survey topographic maps of the study area (Fig. 1). Evaluation of other 1:250000-scale topographic maps has shown that the minimum lake area represented at this scale is about 4 ha (Johnson et al. 1989), although several lakes selected in this study were <4 ha. Most of the study area is contained within the Kenai National Wildlife Refuge, but the refuge boundary does not reflect any strong physiographic differences; consequently, multiple strata were not identified a priori for the Kenai survey.

A systematic sample of 70 lakes was selected from the study area using the procedures specified for an individual stratum in the National Lake Survey (Landers et al. 1988). Lakes were selected from the list of 902 lakes, which was ordered first by latitude and then longitude, by randomly selecting a start and then choosing lakes at fixed intervals. Each lake had an equal probability of being selected. Of the 70 lakes selected, 60 were originally targeted for sampling; 10 were held in reserve as a randomized list to supplement the sample in case of access difficulties or other sampling problems. Six of the 70 mapped lakes selected were found to be wetlands (defined as emergent plants across standing water), two lakes in the wilderness were not sampled because of aircraft restrictions to protect wildlife, and three other lakes were not sampled because of ground access difficulties. The final 59 lakes sampled represent a population of 819 lakes when adjusted for wetlands that were represented as lakes on maps (cf. Landers et al. 1987). Thus, each sample lake represents about 14 lakes in the population of lakes shown at 1:250000 scale on the Kenai Peninsula.

Lakes were sampled during August 15–23, 1988, using floatplanes, helicopters equipped with pontoons, and boats. Lake samples were collected from a depth of 1.5 m using an acrylic Van Dorn (6 L) sampler near the center of each lake. Two aliquots were collected from the sample: a 50-mL nonaerated sample collected from the side of the Van Dorn sampler using a 50-mL plastic syringe secured to a Leur-Lok® fitting for pH measurements (Morris et al. 1986) and a 4-L sample filled into

TABLE 1. Major ion chemistry of lakes on the Kenai Peninsula sampled by USEPA in August 1988. Lakes are sorted in order of ascending alkalinity.

Lake name	Lake ID	Latitude (deg, min, s)	Longitude (deg, min, s)	Area (ha)	Elevation (m)	pH	Alkalinity (μeq/L)	Conductivity (μS)	Silica (μg/L)	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	NH_4^{+} (μeq/L)	SO_4^{2-}	Cl^{-}	NO_3^{-}	DOC (mg/L)
No name	51	60 27 27	151 07 30	4.4	60	5.53	20	13.0	<40	21	25	39	25	6.2	8	47	3.4	13.2
Taiga	05	60 54 41	150 26 56	41.8	56	6.56	26	11.7	<40	17	27	34	14	<0.4	26	37	<0.4	2.1
No name	36	60 43 27	150 30 10	7.9	83	6.31	30	7.1	<40	11	18	29	14	<0.4	3	30	<0.4	4.2
No name	39	60 40 16	150 42 33	7.1	88	6.42	32	7.2	<40	20	18	24	11	<0.4	1	26	<0.4	4.6
No name	54	60 34 17	150 56 29	3.5	67	5.92	32	7.6	<40	19	21	25	16	1.1	2	23	<0.4	13.9
No name	20	60 44 50	150 24 44	5.2	83	6.55	32	7.9	<40	13	19	26	23	<0.4	1	26	<0.4	4.4
No name	46	60 41 46	151 18 14	3.9	34	6.14	32	11.9	<40	18	24	49	21	<0.4	5	54	<0.4	4.0
Birch Tree	10	60 51 34	150 23 54	43.3	67	6.64	36	8.7	<40	17	23	33	11	0.5	5	36	<0.4	3.7
No name	49	60 36 13	150 48 09	20.9	88	6.41	38	10.6	<40	22	30	35	17	<0.4	6	32	<0.4	6.8
Moon	62	60 52 47	150 21 51	47.2	59	6.71	38	13.0	<40	27	33	47	15	<0.4	28	42	<0.4	3.6
Vixen	19	60 43 54	150 25 34	55.2	82	6.81	40	8.7	<40	17	24	30	14	<0.4	3	32	<0.4	3.4
No name	37	60 42 04	150 22 46	19.4	71	6.64	44	10.8	<40	27	25	36	17	<0.4	1	36	<0.4	5.4
Pine	27	60 42 58	151 17 32	6.0	33	6.36	44	10.9	<40	21	26	54	11	<0.4	5	51	<0.4	4.4
Aqua Linda	58	60 19 54	151 13 44	6.2	35	6.63	44	11.4	<40	24	26	42	15	0.07	3	41	2.1	7.1
No name	43	60 41 29	151 02 10	18.7	28	6.58	46	10.5	<40	26	27	48	2	0.06	1	46	<0.4	4.1
No name	06	60 55 13	150 40 08	13.1	38	6.59	46	12.9	<40	23	36	53	12	<0.4	14	54	<0.4	5.4
No name	12	60 50 57	150 43 06	4.2	54	5.95	47	17.5	53	62	63	67	8	0.6	1	55	<0.4	17.5
Porcupine	35	60 42 13	150 37 15	62.0	73	6.92	48	9.1	<40	24	25	31	17	<0.4	5	33	<0.4	2.8
Laura	28	60 42 58	151 17 32	15.5	34	6.62	48	12.8	<40	26	29	47	11	0.6	10	52	<0.4	4.8
Long	38	60 40 24	150 35 12	27.3	64	6.81	52	10.4	<40	28	29	34	20	<0.4	11	31	<0.4	5.4
No name	22	60 44 28	150 39 05	3.5	54	6.96	52	14.3	120	56	51	46	14	<0.4	2	35	<0.4	13.2
No name	04	60 56 27	150 19 56	7.3	48	6.60	60	20.7	<40	45	65	77	22	<0.4	25	74	<0.4	6.9
No name	23	60 44 34	150 53 49	2.9	54	6.33	61	18.0	<40	49	64	69	18	<0.4	3	53	<0.4	11.8
No name	08	60 53 29	150 26 23	23.5	53	6.63	64	14.7	<40	46	50	46	4	0.6	1	40	1.1	10.7
No name	13	60 48 17	150 56 37	15.8	33	6.49	68	20.6	<40	61	51	88	11	1.1	2	82	1.1	12.2
No name	59	60 16 49	151 20 26	4.9	63	6.80	82	20.8	<40	51	52	84	21	<0.4	9	74	<0.4	6.9
No name	17	60 46 28	150 24 44	9.7	83	6.88	84	13.6	<40	51	41	44	18	0.6	1	30	<0.4	4.8
No name	16	60 48 17	150 29 59	4.8	78	6.67	84	14.1	<40	47	44	42	21	<0.4	1	36	<0.4	5.1
No name	48	60 37 00	150 52 53	2.9	98	6.77	86	16.5	<40	51	42	45	33	<0.4	1	42	<0.4	6.5
No name	52	60 35 37	150 41 34	8.8	54	6.82	96	16.8	<40	83	57	47	9	<0.4	1	31	<0.4	11.1
No name	55	60 31 57	151 02 46	2.2	59	6.89	98	18.8	<40	56	53	66	23	<0.4	1	58	<0.4	4.8
No name	47	60 37 11	150 58 18	1.9	68	6.94	102	15.0	<40	47	44	38	31	<0.4	1	30	<0.4	9.9
No name	07	60 53 29	150 34 07	5.2	62	6.45	104	23.2	800	110	83	66	11	<0.4	9	51	<0.4	13.8
No name	63	61 00 26	150 22 01	11.5	34	6.96	106	30.4	<40	69	81	126	25	<0.4	23	125	<0.4	5.0
No name	64	60 42 54	151 16 43	9.2	33	6.93	108	20.7	<40	66	52	79	17	0.9	7	62	<0.4	4.3
No name	67	60 46 04	150 11 51		67	7.02	109	19.3	<40	86	54	64	14	0.6	1	41	1.2	9.7
No name	31	60 41 27	150 53 06	11.4	44	6.99	144	24.0	<40	115	64	58	20	0.6	1	51	<0.4	9.8
No name	65	60 50 08	150 04 42	5.1	62	7.17	154	25.8	124	121	80	72	11	0.6	3	50	2.0	10.0
No name	03	60 58 08	150 23 28	12.1	41	7.20	190	30.6	<40	110	79	85	24	0.6	5	85	<0.4	7.3
No name	60	60 15 30	151 23 01	5.8	68	6.63	194	41.8	<40	97	142	152	31	0.7	3	146	<0.4	10.7
Anertz	32	60 43 00	150 46 45	12.9	74	7.39	200	27.2	<40	147	76	53	16	<0.4	4	36	<0.4	5.5
No name	41	60 39 53	150 53 22	3.8	68	7.01	216	31.6	<40	150	99	70	33	<0.4	6	46	<0.4	8.6
No name	34	60 42 38	150 39 10	10.7	63	7.14	222	28.3	171	164	83	57	16	<0.4	1	30	<0.4	7.5
Derks	56	60 31 46	150 57 59	13.3	48	6.96	222	31.7	845	126	95	80	25	0.6	2	57	<0.4	8.9
No name	53	60 35 42	150 57 35	3.3	63	7.16	236	29.1	122	110	96	88	24	0.6	1	41	<0.4	6.9
No name	26	60 46 01	151 13 48	6.6	47	7.04	262	40.1	<40	152	110	147	26	<0.4	3	108	<0.4	7.3
No name	11	60 51 46	150 38 36	35.6	53	7.27	272	36.8	238	207	110	72	10	0.7	4	49	<0.4	12.4
No name	25	60 44 44	151 05 49	16.9	14	7.65	726	74.2	1270	403	196	179	25	<0.4	4	92	<0.4	6.8
No name	45	60 41 23	151 14 17	9.1	29	7.14	855	91.2	4880	594	223	160	21	1.6	7	74	<0.4	4.4
No name	42	60 39 22	150 55 43	3.7	54	7.35	903	93.0	6120	584	299	146	28	<0.4	1	46	<0.4	6.3
No name	02	60 68 48	150 24 37	14.6	31	7.67	905	112.8	2395	609	301	219	33	0.5	45	189	<0.4	6.6
Beaver Pond	50	60 36 17	150 41 08	18.6	54	7.79	979	96.7	1760	753	237	104	18	0.6	6	24	<0.4	6.6
No name	14	60 47 25	150 45 46	16.9	67	8.09	1007	98.6	2340	669	233	119	33	0.6	31	52	<0.4	5.4
No name	33	60 42 27	150 40 59	10.2	58	7.95	1189	121.2	1540	896	305	146	34	<0.4	11	40	<0.4	5.6
Timber Lost	29	60 41 52	151 13 03	46.5	17	7.87	1213	118.4	8670	813	318	184	25	0.6	1	63	<0.4	5.3
Moose Pasture	18	60 47 55	150 07 52	22.0	58	8.88	1297	128.0	1630	999	309	112	15	0.6	37	61	<0.4	6.0
Duckbill	40	60 39 42	150 41 08	9.3	58	7.59	1405	138.6	1920	1078	353	147	35	0.7	3	50	<0.4	6.0
Hat	15	60 46 42	150 33 46	15.7	56	7.80	1500	146.0	4990	1173	346	141	37	<0.4	1	39	<0.4	5.5
Pan	21	60 46 26	150 03 44	4.1	56	7.70	1738	167.9	7590	1367	389	154	33	0.9	1	35	<0.4	6.4

TABLE 2. Population statistics for lakes on the Kenai Peninsula, Alaska ($n = 59, \hat{N} = 819$). Nonmarine concentrations are denoted with an asterisk.

Parameter	Percentiles				
	0	25th	50th	75th	100th
Lake area (ha)	1.8	4.9	9.5	16.8	62.0
Lake elevation (m)	14	44	58	67	98
pH (unequilibrated)	5.53	6.59	6.88	7.18	8.88
Alkalinity (μeq/L)	20	46	96	236	1740
Conductivity (μS)	8.3	13.4	20.8	40.2	175
DOC (mg/L)	2.2	4.8	6.4	9.7	17.4
Ca^{2+} (μeq/L)	11	26	61	152	1367
Mg^{2+} (μeq/L)	18	29	54	110	389
Na^{+} (μeq/L)	24	42	64	104	219
K^{+} (μeq/L)	2	14	18	25	37
NH_4^{+} (μeq/L)	<1	<1	<1	<1	3
SO_4^{2-} (μeq/L)	1	1	3	7	45
Cl^{-} (μeq/L)	23	35	46	57	189
NO_3^{-} (μeq/L)	<1	<1	<1	<1	3
$RCOO^{-}$ (μeq/L)[a]	22	47	63	95	159
Al_T (μg/L)	<25	<25	<25	<25	231
Silica (μg/L)	<40	<40	<40	240	8670
C_B* (μeq/L)	27	47	99	194	662
C_A* (μeq/L)	0	0	0	3	31
Color (PCU)	5	10	20	30	100

[a]Computed using the method of Oliver et al. (1983).

a collapsible plastic container (Cubitainer®) for all other analyses.

Field measurements included Secchi disk transparency and profile measurements of temperature and conductivity. Samples were stored in coolers and returned to a field laboratory at the U.S. Fish and Wildlife Service Kenai Refuge headquarters in Soldotna for measurement of closed-system pH (unexposed to the atmosphere), true color, and conductivity. Samples were filtered in the field laboratory using Gelman® 0.45-μm membrane filters. All samples were stored in refrigerators and shipped in coolers via overnight courier to the USEPA laboratory in Manchester, Washington, for additional analyses.

Analytical protocols were similar to those used in the National Lake Survey (Landers et al. 1987), with the major exceptions being that alkalinity in the Kenai study was measured using the double-end point method (USEPA method 310.1, 7.2) instead of a Gran titration, and only total Al was measured in the Kenai Survey. Quality assurance procedures included written field and sampling processing procedures, and the use of 12 deionized water blanks, 10 field duplicates, and nine natural lake water performance evaluation samples to estimate contamination, precision, and bias, respectively. These estimates were compared with our objectives for data quality, as described in Landers et al. (1987), and were within the acceptable range.

Alkalinity calculated from the charge balance ($Alk_c = C_B - C_A$ where $C_B = Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}$ and $C_A = SO_4^{2-} + NO_3^{-} + Cl^{-}$) and the measured double-end point alkalinity were strongly correlated ($r^2 = 0.995$), but calculated alkalinity values were consistently greater than measured alkalinity by an average of 31 μeq/L for lakes with alkalinity <100 μeq/L. This disparity is not unexpected for dilute waters with moderate concentrations of organic anions (Sullivan et al. 1989) and largely disappears when an estimate of organic anions based on Oliver et al. (1983) is subtracted from alkalinity calculated using $C_B - C_A$. Sea-salt-corrected ion concentrations, denoted with an asterisk, were computed from marine ratios of ions (Holland 1978), assuming that Cl^{-} was derived entirely from marine aerosols.

Results and Discussion

Lake Chemistry

The physical and chemical characteristics of the 59 sample lakes are shown in Table 1. Summary statistics for the population of Kenai lakes are presented in Table 2. The sample lakes are generally small seepage lakes ranging in surface area from 1.8 to 62 ha with a median size of 9.5 ha. Two large glacier-fed lakes on the peninsula, Skilak (100 km^2) and Tustumena (300 km^2), are not in the sample frame. The sample lakes are all <100 m above mean sea level within a range in altitude of only 84 m, despite being distributed across 4000 km^2.

We attempted to identify patterns in lake chemistry and to associate the patterns with watershed characteristics. The lakes exhibit a wide range in conductivity (8–175 μS), reflecting diverse chemical composition. The pH and alkalinity values shown in Fig. 2 illustrate several important features of the lakes: only three lakes had pH values <6, none of the lakes had alkalinity <0, and the sampled lakes fell into two classes, those with alkalinity <300 μeq/L (solid circles) and those >700 μeq/L (open circles). Such a strong partitioning of randomly selected lakes into nonoverlapping alkalinity classes (Table 2) is unusual and, to some degree, reflects the uncertainty associated with a probability survey. Nonrandom surveys of 47 large lakes on the refuge for evaluation of fish habitat by the U.S. Fish and Wildlife Service (1983–90) included nine lakes with alkalinity between 300 and 700 μeq/L. The differences in results of these two surveys may be explained by the fact that our study was randomized to describe the characteristics of the entire defined lake population ($n = 819$) whereas the fisheries study was nonrandom,

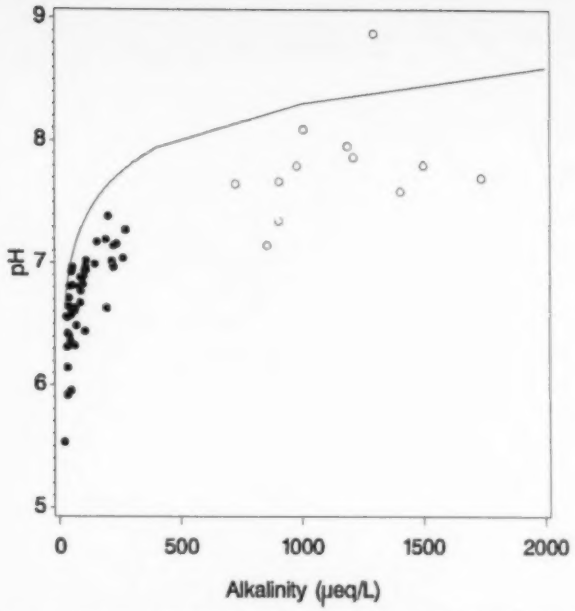

FIG. 2. pH versus alkalinity for 59 sample lakes on the Kenai Peninsula. The line represents the expected distribution for lakes with a P_{CO_2} value of $10^{-3.5}$ atmospheres. Solid circles represent the Group I lakes and open circles represent the Group II lakes.

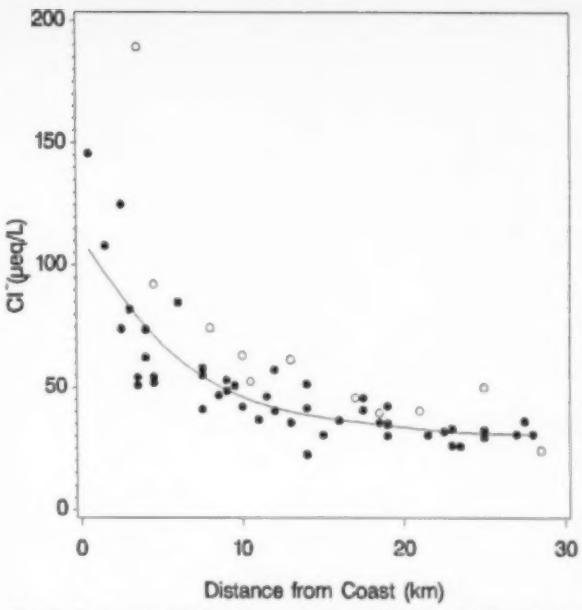

FIG. 3. Cl^- concentrations in the 59 sample lakes on the Kenai Peninsula versus distance from the Cook Inlet. The line is a cubic spline fit for these data (SAS Institute, Inc., 1988). Solid circles represent Group I lakes and open circles represent Group II lakes.

targeting specific lakes of interest for fish habitat. The characteristics of the two studies clearly differed; the median lake size of the fishery study was 49.6 ha, about five times the median lake area of our defined population (9.5 ha).

The uncertainty associated with our randomized survey design may be better demonstrated with an example. Given that we sampled 59 lakes from a population frame of 819, the 95% confidence limit on the possible size of an unsampled subpopulation is 39 lakes. In other words, although we sampled no lakes in the 300–700 μeq/L alkalinity range, we could expect that as many as 39 such lakes may be present in the population. Thus the probability survey is consistent with the extant data.

We defined two groups of lakes based on the distribution shown in Fig. 2, where Group I comprises lakes with alkalinity <300 μeq/L and Group II lakes with alkalinity >700 μeq/L. The two lake groups also differed significantly with respect to most other chemical attributes (Table 3). Classification of the lakes with nonhierarchical cluster analysis (FASTCLUS; SAS Institute, Inc. 1988), using major ions, pH, silica, and DOC, resulted in the same partitioning into two primary clusters. The exceptions are Lake No. 25, the lowest alkalinity lake in Group II, which grouped with Derks Lake (No. 56) and Lake No. 07, both of which had the highest silica concentrations among the Group I lakes. Of the 47 Group I lakes, 43 are seepage lakes whereas 6 of the 12 Group II lakes are drainage lakes based on 1:24000-scale topographic maps. Thus, hydrologic classification derived from topographic maps provides a general, but imprecise, indication of the lake chemistry for the Kenai Peninsula. Trophic state classification of the lakes using P was not useful in this survey because all but one of the lakes had total P concentrations less than the detection limit of 7 μg/L.

As expected, one of the most prominent spatial patterns evident in the data was the relationship between distance from the coast and Cl^- concentrations in the lakes (Fig. 3). Lakes within 10 km of the coast had Cl^- concentrations generally above 50 μeq/L, with values declining with increasing distance from the coast. The relationship of the Cl^- concentrations with distance from the coast observed on the Kenai Peninsula appears similar to those observed in Maine (Mairs 1967) and the northeastern United States (Sullivan et al. 1988a; Baker et al. 1990), but the northeastern lakes had higher Cl^- concentrations, presumably because of higher evaporation rates and orientation of lakes with respect to local wind patterns.

Sodium did not decrease with distance from the coast in a pattern similar to that observed in Fig. 3 for Cl^-. In most lakes, the Na:Cl ratio was ≥0.86, the ratio in seawater, indicating that additional Na^+ was being weathered in some watersheds (Fig. 4). An alternative explanation for this pattern is that Cl^- is being retained in some of the watersheds, but the uniformity of the Cl^- pattern suggests that the magnitude of any Cl^- retention is likely to be small (e.g., <10 μeq/L) relative to the differences between Na^+ versus Cl^-. This is particularly evident in the higher-alkalinity lakes (Fig. 4, open circles), where the mean watershed contribution of nonmarine Na^* was 96 μeq/L, compared with only 15 μeq/L in the low-alkalinity lakes (Table 3). No correlation was observed between the Na^* concentrations and alkalinity values or ratios of individual base cations to Na^*. These indicators provided no evidence of retention of neutral salts in the watersheds which can consume alkalinity (Wiklander 1975). Rather than being retained, Na^+ was an important weathering product from the watersheds of most of these lakes.

Concentrations of base cations other than Na^+ in the lakes showed an equally large disparity between low- and high-alkalinity lake classes. In addition, the relative abundance of

TABLE 3. Mean (and SD) of physical characteristics and surface water chemistry[a] for low-alkalinity (Group I) and high-alkalinity (Group II) lakes, Kenai Peninsula, Alaska. Nonmarine mean concentrations for selected ions (assuming that all Cl^- is marine) are denoted with an asterisk.

Parameter	Group I (alkalinity <300 μeq/L)	Group II (alkalinity >700 μeq/L)	t-test[b] (Prob > |t|)
n	47	12	
Estimated population	652	167	
Lake area (ha)	14.1 (14.7)	15.6 (11.3)	0.6998
Lake elevation (m)	58.9 (17.4)	46.0 (18.0)	0.0399
pH	6.71 (0.37)	7.79 (0.42)	0.0001
Alkalinity (μeq/L)	95 (71.0)	1143 (301)	0.0001
Conductivity (μS)	20.1 (9.5)	122 (27.7)	0.0001
DOC	7.5 (3.6)	5.9 (0.7)	0.0062
Ca^{2+} (μeq/L)	63 (48) 61*	828 (283) 826*	0.0001
Mg^{2+} (μeq/L)	52 (30) 42*	292 (59) 279*	0.0001
Na^+ (μeq/L)	58 (29) 15*	151 (32) 96*	0.0001
K^+ (μeq/L)	7 (7) 16*	28 (7) 27*	0.0003
SO_4^{2-} (μeq/L)	6 (7) 1*	12 (16) 5*	0.1754
Cl^- (μeq/L)	50 (25)	64 (44)	0.3027
Silica (μg/L)	86[c] (162)[c]	3759 (2595)	0.0005
Color (pcu)	28 (25)	19 (6)	0.0329

[a]Concentrations for NH_4^+ and NO_3^- were generally less than the detection limit of 0.4 μeq/L.
[b]t-test on differences between lake groups computed assuming unequal variances.
[c]Computed by scoring the observations measured at their detection limit to 40 μg/L. The mean and standard deviation of the silica for the Group I lakes using a ranking procedure for censored data are 69 and 167 μg/L, respectively.

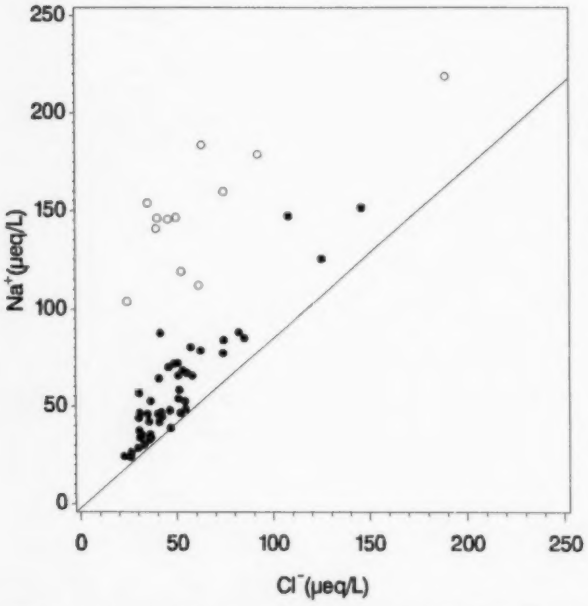

FIG. 4. Na^+ versus Cl^- concentrations in 59 sample lakes on the Kenai Peninsula. The line shows the expected relationship if the concentrations of both ions were derived entirely from marine aerosols. Solid circles represent Group I lakes and open circles represent Group II lakes.

individual ions differed between the groups. The equivalent ratios of the sea-salt-corrected base cations (Ca^*:Mg^*:Na^*:K^*) normalized to K^+ in the Group I lakes (3.8:2.6:0.9:1) differed in several major respects from ratios in the Group II lakes. The Ca^*:Mg^* ratio was 1.5 in the Group I lakes and 3.0 in the Group II lakes. The Ca^*:Na^* ratio in the two lake groups (4.2 and 8.5) also differed by a factor of 2.

These differences in concentrations and ratios of base cations were reflected in the silica concentrations for the two lake groups. Only 8 of the 47 Group I lakes had silica concentrations greater than the estimated detection limit of 40 μg/L; for lakes with alkalinity <100 μeq/L, silica was virtually undetectable. High-alkalinity lakes had Si values ranging from 1270 to 8670 μg/L (Table 1). Although some silica is consumed in biological processes (e.g., Hurley et al. 1985), we believe that it is sufficiently unreactive in these lakes to provide an indication of dominant weathering reactions. Drever (1982) and Stauffer (1990) described some possible scenarios for achieving results similar to those observed here for the high-alkalinity lakes, including weathering of amphiboles. Shoji et al. (1988) found a number of silicate minerals in these soils, including amphiboles, pyroxenes, opaline silica, and feldspar that could serve as sources of silica in the high-alkalinity lakes. The low concentrations of silica in the low-alkalinity lakes are further evidence of the apparent hydrologic isolation of these lakes.

Whereas the concentrations of all major cations differed between the two lake groups, the only anions that differed significantly ($p \leq 0.05$) were bicarbonate (as alkalinity) and organic anions (as DOC) (Table 3). The lakes were almost uniformly low in SO_4^{2-} and NO_3^-. The median [SO_4^{2-}] for all lakes was <3 μeq/L and the sea-salt-corrected median was <0, indicating that SO_4^{2-} was being retained in the watersheds (e.g., Bayley et al. 1986) or was being reduced through in-lake processes (Baker et al. 1986). The presence of extensive wetlands in the study area provides an excellent opportunity for sulfate retention. Seven of the lakes had [SO_4^{2-}] >20 μeq/L. Only six of the study lakes had [NO_3^-] greater than the estimated detection limit of 0.4 μeq/L, although delays in analysis of the samples could have caused a significant loss of NO_3^- in the samples. Differences in [Cl^-] between the two lake groups were not significant ($p \leq 0.05$).

TABLE 4. Major ion chemistry (mean and SD) of groundwater and rivers on the northern Kenai Lowlands (source: Anderson and Jones 1972).

Parameter	Groundwater		River	
	Shallow aquifer	Artesian aquifer	Moose[a]	Swanson[b]
n	8	10	1	1
Depth (m)	17.1 (5.5)	51.8 (16.8)		
pH	7.4 (0.46)	8.0 (0.20)	7.8	7.0
Conductivity (μS)	150 (48)	634 (705)	159	171
Ca^{2+} (μeq/L)	1003 (384)	753 (684)	1148	1048
Mg^{2+} (μeq/L)	510 (584)	469 (321)	183	283
Na^{+} (μeq/L)	213 (57)	4872 (6525)	183	282
K^{+} (μeq/L)	46 (20)	146 (113)	33	46
HCO_3^- (μeq/L)	1393 (459)	3311 (2082)	1606	1688
SO_4^{2-} (μeq/L)	44 (117)	198 (300)	0	20
Cl^- (μeq/L)	102 (48)	2934 (5162)	20	99
Silica (mg/L)	31.8 (9.5)	36.8 (8.1)	15.7	15.7

[a]Sampled November 1, 1967.

[b]Sampled December 2, 1958; data collected from May 30 to September 20, 1988, on the Swanson River showed a mean pH value of 7.6 (0.16), $n = 16$, a mean conductivity of 169 (26) μS, $n = 17$, and an alkalinity of 1638 (279) μeq/L (Kenai National Wildlife Refuge, unpublished data).

Mean DOC concentrations were significantly ($p \leq 0.05$) greater in the low-alkalinity lakes ($\bar{x} = 7.5$ mg/L) than in the high-alkalinity lake group ($\bar{x} = 5.9$ mg/L), although there was greater variability in the low-alkalinity group. Despite the high concentrations of DOC in some lakes, none of the lakes was acidic (alkalinity <0) and the minimum pH measured was 5.5. A number of these lakes receive organic inputs from spodic soils and bog vegetation such as *Sphagnum*, which are common throughout the study area. In addition to the production of organic acids from spodic soils and acidophilic bryophytes, additional organic acids could be derived from the Andic-type soils which have multiple organic horizons. Nevertheless, the organic acid production from soils in the study area is insufficient to overcome the alkalinity production from watershed weathering and in-lake processes.

Lake Hydrology

By examining the patterns in lake chemistry and differences in physical setting, we can make some assessments regarding the likely principle sources of inflow to the various study lakes. For example, the uniformity of moderate DOC values (SD = 0.7 mg/L) in the high-alkalinity lakes is attributed to the likely dominance of surface water inflows to most of the Group II lakes. Several of the high-alkalinity lakes are located in the Swanson River and Moose River drainage systems, and these lakes are expected to be similar in many respects. The lower average concentration of DOC in the high-alkalinity lakes may be attributed, in part, to substantial groundwater discharge to their hydrologic budget, either as direct groundwater discharge to the lakes or as a major component of the surface inflow. DOC concentrations were far more variable in the low-alkalinity lakes, ranging from 2.2 to 17.5 mg/L (SD = 3.6 mg/L). The lower-alkalinity lakes, typically, are seepage lakes situated in hummocky terrain at elevations greater than the high-alkalinity systems. The elevation of the low-alkalinity lakes averages 59 m compared with 46 m above mean sea level for the high-alkalinity lakes; for six pairs of Group I and Group II lakes in proximity to one another, Group I lakes have elevations greater than or equal to Group II lake elevations, with a mean difference of 9.5 m (Table 3). This suggests that the low-alkalinity lakes are elevated above deeper groundwater flowpaths and act as groundwater recharge systems whereas the high-alkalinity lakes are intercepting high-solute groundwater and surface water derived from groundwater.

The strong disparity in major ion chemistry between the two groups of lakes from the Kenai Lowlands also suggests that high-alkalinity lakes receive substantial groundwater discharge. Water chemistry from wells and the two principal rivers draining the study area (Table 4) showed concentrations of most dissolved ions comparable with those observed in this study (Table 2). Although artesian aquifers are present in the Kenai Lowlands, the agreement between the shallow aquifer data and the lake chemistry suggests that discharge from the shallow groundwater aquifer is sufficient to explain the high concentrations in the Group II lakes. The chemistry of the Moose and Swanson rivers, although based on only one sample each, also showed close agreement with the major ion chemistry of the shallow aquifers. The major disparity in the shallow groundwater chemistry is silica, where the river concentrations were only 50% as great as that of the shallow groundwater aquifer.

In an examination of groundwater and lake level fluctuations, Anderson and Jones (1972) noted that lakes located on river systems such as the Moose and Swanson showed little fluctuation in lake levels. Local aquifers and the lakes in glacial drift associated with these shallow aquifers tended to exhibit water elevation declines of 1.2–1.8 m during extended droughts whereas lakes situated in till above the aquifers showed smaller changes in lake stage (e.g., <0.7m). The inference is that lakes with closed basins (i.e., seepage lakes) exhibiting relatively large stage fluctuations are groundwater discharge lakes whereas the closed-basin lakes exhibiting small fluctuations in stage are groundwater recharge lakes. All 12 high-alkalinity lakes had defined stream connections or had substantial wetland areas adjoining the lake shoreline. This suggests to us that these wetlands are groundwater discharge areas, which is consistent with Anderson and Jones (1972), who previously delineated the major wetlands on the Kenai Peninsula as groundwater discharge sites.

Without additional groundwater chemistry and hydrologic data on individual lakes, it is not possible to distinguish unequivocally between differences in mineralogy and percentage of groundwater contribution as causal mechanisms for differences in the low- and high-alkalinity lakes. Indeed, both factors may influence the differences in lake chemistry. Results from simple geochemical models provide support for concluding that the lakes differ because of weathering of different mineralogy. However, groundwater and rivers draining the Kenai Peninsula have high silica concentrations, and yet most of the low-alkalinity lakes lack measurable silica. This suggests that many of the Group I lakes do not receive substantial groundwater input. In addition, there is physical evidence that the low-alkalinity lakes receive little groundwater because of the elevation differences between the low- and high-alkalinity lakes.

Local Airshed and Watershed Perturbations

Although most of the study lakes are located in designated wilderness, there is sufficient industrial and residential development on the Kenai Peninsula that we needed to evaluate the potential for some of these activities to influence the chemistry of the lakes. One of the major concerns in the area is associated with industrial emissions in and near the Kenai Peninsula, particularly in the immediate vicinity of the Nikiski industrial complex (Fig. 1). This concern has prompted several studies in the area, including analyses of trace metal deposition (Severson et al. 1990), vegetation condition (Sullivan et al. 1990), and potential acidification of surface waters (ADEC 1981). Severson et al. (1990) identified elevated concentrations of trace contaminants close to Nikiski (e.g., within 1 to several kilometres) but found no evidence of elevated deposition of trace metals in the Kenai National Wildlife Refuge. Sullivan et al. (1990) measured acute damage to the dominant tree species, white spruce, and noted major changes in understory species composition near Nikiski (e.g., <1 km), but readily observable effects attenuated rapidly within 5 km of the sources. They attributed the vegetation damage to emissions of NO_x and NH_3, which totalled over 22 000 tons annually (ADEC 1990); ambient air concentrations of these N species exceeded 4 ppm near Nikiski in 1990 (ADEC 1990). The study by ADEC (1981) consisted of sampling surface waters from several high-alkalinity lakes and streams in the spring of 1981. Results of this aquatic study were inconclusive because the high-alkalinity waters were well buffered and the analytical methodology was inadequate relative to the study objectives.

Our closest sample lake to Nikiski was 4.5 km from the emission source, and it showed no evidence of chronic acidification. The data from this lake survey are insufficient to evaluate potential impacts to lakes adjacent to industrial emissions, in part, because so few lakes were sampled within 5 km of these emissions. A study of chronic impacts near the Nikiski industrial complex would need to be designed with a sufficient number of lakes close to the sources. Moreover, likely rapid uptake of N in the summer suggests that effects on the lakes would be best evaluated with an intensive study during the spring.

Although current SO_4^{2-} deposition on the Kenai Peninsula is probably elevated slightly above preindustrial levels, we estimate that the annual deposition for most of the study lakes is <5 kg·ha^{-1}·yr^{-1}. The concentrations of SO_4^{2-} in the sample lakes can be explained largely by marine aerosols (Table 3). The low SO_4^{2-} concentrations in these Alaskan lakes and the absence of acidic, low-DOC lakes agree with empirically based predictions of expected acid–base status for areas receiving low SO_4^{2-} deposition (Henriksen et al. 1988; Schindler 1988; Baker et al. 1990).

Another concern to us was the possibility that current or previous drilling activities for fossil fuels in the Kenai Peninsula could have altered the chemistry of some of the lakes. An examination of the location of drilling activities by the U.S. Fish and Wildlife Refuge staff indicated that most of the study lakes were >2 km from major drilling locations, and only a few lakes were within 1 km of such sites. The spatial patterns of documented drilling exploration and high-alkalinity lakes showed no apparent association.

Some of the study lakes had low to moderate residential development, but concentrations of P and N were so low that no impact was evident from the survey data. We conclude that the chemical patterns observed in our lake survey are not artifacts of anthropogenic disturbance.

Implications for Lake Surveys

This survey on the Kenai Peninsula provides resource management agencies with quantitative estimates of lake chemistry that can be applied to decisions in fisheries and wildlife management and provide a reference for evaluating future changes in lake chemistry. One of the interesting results of this survey is the major difference in chemistry for lakes within a relatively small study area and the same physiographic unit.

A key finding from this survey relevant to survey design is that strata often used for identifying relatively homogeneous populations of lakes are difficult to identify a priori on the basis of characteristics typically employed for such activities (e.g., soils, surficial geology, bedrock geology, physiography) and at the scales at which these resources are typically mapped. Although we expected to encounter lakes with a moderate range of chemical characteristics, there were few hints from the physiography of the northern Kenai Peninsula that we would identify two classes of lakes. A selective survey of lakes on the peninsula showed that there are some lakes with chemical attributes intermediate between these two classes, but the randomized design of our survey allowed for not sampling a subpopulation as large as 5% of the lake population.

Surveys such as this provide a basis for relating results from intensive studies of single lake systems to regional populations of lakes or for selecting individual study lakes intended to be representative of a lake population. For example, one of the lakes that has been the focus of previous investigations on the Kenai Peninsula is Bernice Lake (ADEC 1981). The alkalinity of 760 μeq/L for Bernice Lake places it in the Group II lakes, representing only 20% of the lakes in the study area. The emphasis on studying Bernice Lake is understandable in that it is located within a state park; however, further study of Bernice Lake alone provides little information regarding the likely response of the majority (and most sensitive) of lakes on the Kenai Peninsula to atmospheric emissions. The probability survey of the Kenai lakes also complements targeted roadside surveys used by the refuge to characterize conditions in the larger lakes on the Kenai Peninsula (U.S. Fish and Wildlife Service 1983–90).

Summary and Conclusions

A systematic survey of 59 lakes in August 1988 was used to characterize a population of over 800 lakes in the northwestern

Kenai Peninsula. Despite the widespread presence of acidic soils in the study area, no acidic lakes were sampled and the concentrations of SO_4^{2-} and NO_3^- were generally near detection limits. The only strong spatial pattern in lake chemistry noted within the study area was the association between Cl^- and distance from the coast. Most of the cation concentrations in the lakes, including Na^+, were associated with watershed sources. Although DOC concentrations were high in some lakes (max. = 17.5 mg/L), all lakes had sufficient alkalinity (min. = 20 μeq/L) to maintain pH values >5.5. The acid–base chemistry of the Kenai lakes is similar to that expected on the basis of synoptic surveys in the conterminous United States (Linthurst et al. 1986; Landers et al. 1987) where lakes with strong mineral acidity were found only in areas receiving substantial amounts of SO_4^{2-} deposition (Sullivan et al. 1988b; Baker et al. 1991a).

The population of Kenai lakes consists of two lake subpopulations: one of low-alkalinity, low-silica lakes ($\hat{N}$ = 637) and one of high-alkalinity, high-silica lakes ($\hat{N}$ = 182). The former group consists of lakes with alkalinity <300 μeq/L and ranging in DOC from 2.2 to 17.5 mg/L. The median silica concentration in these lakes was less than the estimated detection limit of 40 μg/L. The high-alkalinity class comprised lakes with alkalinity >700 μeq/L (mean = 1143 μeq/L), relatively uniform DOC concentrations (mean = 5.9 mg/L), with high silica concentrations (mean = 3759 μg/L). The low-alkalinity lakes probably receive little groundwater or surface water inputs, and these contributions are probably from very shallow sources adjacent to the lake. In contrast, the high-alkalinity lakes probably receive greater quantities of groundwater from deeper aquifers or surface inputs consisting, in part, of discharge from these aquifers. The suspected differences in hydrologic flowpaths between these two groups of lakes appear to be associated with relatively minor differences in lake elevation, where the high-alkalinity lakes are located in or near groundwater discharge areas and the low-alkalinity lakes are situated at slightly higher elevation in recharge areas.

In addition to providing management agencies with quantitative information on lake chemistry, the results of this study may provide a model for conducting investigations among Alaska's 3 million lakes. Regional surveys such as this provide a basis for selecting lakes for more intensive study and for placing other studies in the context of defined populations of lakes.

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Influence of Age-Selective Surveys on the Reliability of Stock Synthesis Assessments

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We used Monte Carlo simulations to examine the reliability of assessments following a method of separable catch-at-age analysis (Methot's stock synthesis approach). Our simulations were based on the widow rockfish (*Sebastes entomelas*) fishery off the west coast of the continental United States. We designed the study to examine how the performance of the method depended upon the age-specific selectivity pattern of a survey of relative abundance. When survey selectivity increased asymptotically with age, stock synthesis estimates of final year biomass (and most other parameters) tended to be closest to the correct value. When the survey concentrated on young fish the estimates were least reliable. When the survey selectivity was "dome-shaped," concentrating on intermediate-aged fish, estimates were somewhat less precise (and less accurate) than for the asymptotic pattern but much more precise than for the survey concentrating on young fish. Predictably, estimates of recruitment and biomass were better at a higher fishing mortality rate and when the magnitude of survey error was lower. Generally, mean errors in estimated biomass and recruitment were positive, while median errors were close to zero. This pattern is due to transformation bias associated with a lognormal error structure.

Nous avons utilisé des simulations de Monte-Carlo afin de déterminer la fiabilité d'évaluations réalisées par analyse à composantes séparables des prises selon l'âge (synthèse des stocks de Methot). Nos simulations étaient fondées sur la pêche de la veuve (*Sebastes entomelas*) réalisée au large de la côte ouest des États-Unis. Nous avons conçu l'étude de façon à déterminer dans quelle mesure le rendement de la méthode dépendait de l'allure de la sélectivité spécifique quant à l'âge d'un relevé de l'abondance relative. Lorsque la sélectivité du relevé augmentait de façon asymptotique avec l'âge, l'estimation par synthèse du stock de la biomasse de dernière année (et de la plupart des autres paramètres) tendait à se rapprocher le plus de la valeur réelle. L'estimation la moins fiable était obtenue lorsque le relevé portait surtout sur les poissons les plus jeunes. Lorsque la sélectivité du relevé correspondait à la forme "en dôme" et portait donc sur les poissons d'âges intermédiaires, l'estimation était quelque peu moins précise (et moins exacte) que celle du relevé d'allure asymptotique, mais demeurait plus précise que celle du relevé portant surtout sur les jeunes poissons. Comme on peut s'y attendre, l'estimation du recrutement et de la biomasse était meilleure lorsque le taux de mortalité par pêche était plus élevé et que l'erreur du relevé était plus faible. De façon générale, l'erreur moyenne de la biomasse et du recrutement estimé était positive tandis que l'erreur médiane se rapprochait de zéro. Ce phénomène s'explique par des erreurs de transformation liées à un biais de structure lognormale.

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Separable age-structured population models are widely used to analyze fishery catch-at-age data in order to estimate recruitment, fishing mortality rates, and stock abundance. These models assume that the fishing mortality rate can be separated into a product of year and age effects (Doubleday 1976). In most instances, a wide range of solutions to a separable model can fit a set of fishery age-composition data about equally well (Doubleday 1976; Deriso et al. 1985; Shepherd and Nicholson 1986). Thus, for reasonably accurate estimates of abundance, separable models require auxiliary data such as information about abundance from trawl surveys or measures of fishing effort (Doubleday 1976; Deriso et al. 1985; Kimura 1990; Methot 1990).

In this paper, we examine the performance of a separable model when survey data on the relative abundance of a stock are available. Our objective is to compare the merits of surveys with different types of age-specific selectivity patterns. Surveys that cover the habitat occupied by adult fish sometimes have selectivity increasing to an asymptote (Rollefsen 1953). Other surveys that use selective gear (e.g., gill net or hook-and-line) or concentrate in a subhabitat occupied predominantly by certain ages may have either a dome-shaped selectivity with peak

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selectivity at an intermediate age (e.g., Hamley 1975; Methot and Hightower 1990; Ralston 1990) or peak selectivity on recruits (young fish) (Methot and Hightower 1990; Wyllie Echeverria et al. 1990). The separable model we use belongs to Methot's (1990) family of stock synthesis models, which are widely used in assessing fish stocks off the west coast of North America (PFMC 1990). We fit the model to simulated data based on the biology of widow rockfish (*Sebastes entomelas*), which supports a significant trawl fishery off the west coast of the continental United States. This species, like other *Sebastes*, is relatively long-lived, with a maximum reported age of 50 yr in California and an estimated natural mortality rate of 0.15. Although we used the biology of widow rockfish as a basis for our modeling, our goal was to determine general results regarding the performance of the stock synthesis model, not to mimic the widow rockfish fishery exactly.

Simulation Design and Methodology

The basic stock synthesis approach is to invoke a dynamic model both of the fishery and of the process by which data are generated and then fit this model to the available data (Methot 1990). The synthesis model estimates a suite of parameters and uses these along with prespecified constants to calculate fitted data values. These fitted or "synthetic data" are then compared with the observed data in likelihood equations, and a search is done for the set of parameter estimates that maximizes the likelihood.

We examined the performance of the stock synthesis model by Monte Carlo simulation. We did 200 simulations for each of the 18 combinations of age-specific selectivity patterns for the survey, average fishing mortality rates during the simulations, and levels of survey error.

We considered surveys with (1) increasing asymptotic selectivity, (2) dome-shaped selectivity, and (3) maximum selectivity for young fish (recruits) (Fig. 1). For each survey selectivity pattern, we used two average levels of fishing mortality (low: mean fishing mortality rate at age 10 (F) = 0.075 (half the natural mortality rate, i.e., $M/2$ and high F = 0.30 ($2M$)) and three levels of survey error: low (CV = 10%), medium (CV = 25%), and high (CV = 50%). Note that all cases started in an unfished condition, which is a reasonable portrayal of the widow rockfish fishery prior to 1980. The range of error in surveys is in accord with typical estimates of survey precision (e.g., Rivard 1981; Pope 1983; Pope and Gray 1983; Weinberg et al. 1984).

We first describe the synthesis model used in our simulations, then describe how we generated simulated data sets, and lastly turn to details on how the "best fit" of the synthesis model to the simulated data sets was achieved for each run. With only minor exceptions discussed below, the model fitted in the simulated assessments (i.e., the synthesis model) was the same as the model we used to produce the simulated data sets. That is, our evaluation of the stock assessment methodology did not include appreciable process error.

Stock Synthesis Model

Following after Lenarz and Hightower's (1988) assessment of the widow rockfish fishery, nine age groups of fish were included in the synthesis model (age 5 through 13+), and we followed the population for a 15-yr time period. The numbers of fish of each age group, starting with age 6, present at the beginning of the first year ($N_{a,1}$, a = 6, ..., 13) and the number of age 5 fish recruiting at the beginning of each year ($N_{5,y}$, y = 1, ..., 15) were estimated as formal parameters as described below. Given estimates of initial numbers and recruitment, the numbers of age a fish at the beginning of each year y after the first ($N_{a,y}$, a = 6, ..., 12; y = 2, ..., 15) were simply the survivors from the previous year:

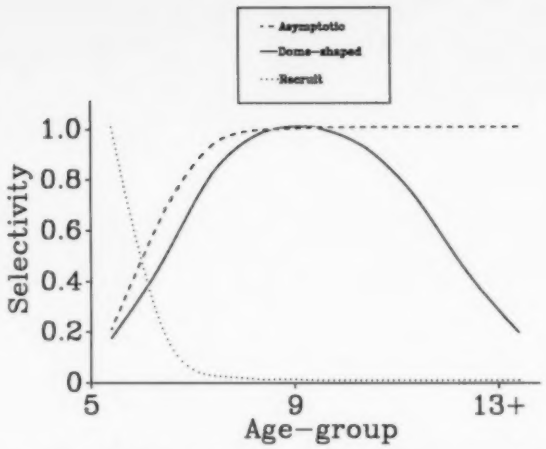

FIG. 1. Selectivity functions for the survey. Note that the dome-shaped function was also the fishery selectivity pattern. Parameters used to generate these functions are given in Table 1. Selectivity has been renormalized, for this presentation only, so that the maximum value is 1; this allows the selectivity patterns for the different surveys to be presented on the same scale.

$$(1)\quad N_{a,y} = N_{a-1,y-1} \exp(-\{M + F_{a-1,y-1}\})$$

where M is the natural mortality rate and $F_{a,y}$ is the fishing mortality rate for age a fish in year y. For the 13+ category, numbers in each year after the first ($N_{13,y}$) were given by

$$(2)\quad N_{13,y} = N_{12,y-1} \exp(-\{M + F_{12,y-1}\}) + N_{13,y-1} \exp(-\{M + F_{13,y-1}\})$$

which were simply the survivors of the 13+ category plus the surviving 12-yr-olds from the previous year. Thus, numbers-at-age, excluding the initial age composition and recruitment each year, were not estimated as parameters, but instead were calculated from the initial age composition ($N_{a,1}$, a = 6, ..., 13), recruitments ($N_{5,y}$, y = 1, ..., 15), age- and year-specific fishing mortality rates ($F_{a,y}$, a = 5, ..., 13; y = 1, ..., 15), and the natural mortality rate (M).

$F_{a,y}$ was modeled as being separable:

$$(3)\quad F_{a,y} = F_y s_{f,a}$$

where $s_{f,a}$ was the fishery (f) selectivity on age a fish and F_y scaled overall fishing mortality in year y. In the model, $s_{f,10}$ was set equal to 1, so that F_y equals $F_{10,y}$, the fishing mortality rate on age 10 fish in year y. A restriction of this type is necessary to parameterize uniquely the age and year effects (Doubleday 1976). We chose to normalize to selectivity at a constant age, rather than to the age of estimated maximum selectivity, as is often done, so that F_y would have a constant definition. Thus, selectivity for some ages could exceed unity.

Age-group-specific fishery selectivity was assumed to follow a double-logistic function (Table 1; Fig. 1). The double-logistic

TABLE 1. Parameters and values used by the stock synthesis model (SSM) and by the simulation model. Some of these values were estimated by the SSM as formal parameters (E), others were assumed known (K), and others were determined by assumed constraints (C) (see text). Note that "known" values used for the SSM were the same as the values used in the simulations.

Parameter	Description	Treatment by SSM	Values used by simulation
F_y	Fishing mortality multiplier for year	C	From lognormal distribution with CV = 30% and mean of 0.075 or 0.30·yr^{-1}
$N_{5,y}$	Recruitment at age 5 in year y	E	From lognormal distribution; mean = 17.8M, variance = 110.3M
$N_{a,1}$	Initial numbers-at-age	E	Based on distribution for recruitment parameters
$\alpha_{f,i}$, $\beta_{f,i}$, $\alpha_{f,d}$, $\beta_{f,d}$	Fishery selectivity parameters	E	−9, 1.46, 13.28, −1.14
$\alpha_{s,i}$, $\beta_{s,i}$, $\alpha_{s,d}$, $\beta_{s,d}$	Survey selectivity parameters	E	
	Recruit		−1, 0.1, 14, −2.655
	Dome-shaped		−9, 1.46, 13.28, −1.14
	Asymptotic		−11.39, 2, 13.28, −0.001
q	Survey proportionality constant	C	1
M	Natural mortality rate	K	0.15·yr^{-1}
σ_s	SD of log survey catch	K	Produces CV = 10, 25, or 50%
σ_c	SD of log fishery catch	K	Produces CV = 1%

function is flexible and can be used to model a wide variety of selectivity patterns, including nearly knife-edged, dome-shaped, and asymptotic (Methot 1990). This function is composed of two logistic functions, one increasing and the other decreasing. The product of the two logistic functions, after re-scaling, was selectivity:

$$(4)\quad s_{f,a} = \frac{[1/\{1+\exp(-(\beta_{fi}\,a + \alpha_{fi})\}]\,[1/\{1+\exp(-(\beta_{fd}\,a + \alpha_{fd})\}]}{\lambda_{10}}$$

where α_{fi}, α_{fd}, β_{fi}, and β_{fd} are the parameters of the increasing (i) and decreasing (d) logistic functions and λ_{10} is equal to the value of the numerator of the equation for a (age) = 10. α_{fi}, α_{fd}, β_{fi}, and β_{fd} were estimated as formal parameters, while the overall scalers of fishing mortality, F_y, y = 1, ..., 15, were determined by constraints as described below. (Note that the selectivity of the 13+ group was assumed equal to the value predicted by equation 4 for a = 13.)

The last determinant of population dynamics was the natural mortality rate (M), which was fixed at a value of 0.15 based on prior information (Hightower and Lenarz 1986).

We now turn to how the synthesis model calculated its synthetic data to compare with observed data. We assumed that the observed data for each 15-yr period consisted of fishery age-composition data, total biomass of fishery catch during each year, annual survey age composition data from subsamples of the survey catch, and catch per unit effort (CPUE), a relative annual index of biomass available to the survey gear.

The numbers of each age caught by the fishery during each year were assumed by the synthesis model to follow the Baranov catch equations:

$$(5)\quad \eta_{f,a,y} = N_{a,y}\,F_{a,y}\,[1\text{-}\exp\{-M + F_{a,y})\}]/[M + F_{a,y}].$$

The synthesis model treated the fishery catch data as consisting of two independent pieces of information: (1) the proportion of the total numbers caught each year that fell into each age-group (i.e., the age-composition data), which were directly calculated from the $\eta_{f,a,y}$'s, and (2) the total biomass caught ($C_{f,y}$). Catch biomass was calculated simply as the sum over ages of the product of numbers caught at each age (from equation 5) and the weight at each age. The weight-at-age relationship was assumed to be known a priori, and equations developed by Lenarz (1987) and Phillips (1964) for widow rockfish were used to define this relationship.

Like the fishery catch data, the survey data were treated as containing two independent types of information for each year: (1) the proportions caught by the survey at each age (the age-composition data) and (2) a CPUE value representing an index of biomass available to the survey gear. The survey was assumed to have an insignificant effect on the stock size and to have occurred after recruitment for a given year, but before fishing or natural mortality. The synthesis model calculated the proportions-at-age caught by the survey in year y, the $P_{s,a,y}$'s, as proportional to the product of the model's current values for abundance of each age and the selectivity of the survey for the corresponding age ($s_{s,a}$):

$$(6)\quad P_{s,a,y} = s_{s,a}N_{a,y} / \Sigma_i\, s_{s,i}N_{i,y}\,.$$

In our application of the synthesis model, the survey selectivity pattern was defined by a double logistic function of age, just as the selectivity of the fishery was. The four parameters defining the function were estimated as formal parameters (Table 1). The CPUE index was fit by the synthesis model by assuming it to be proportional to the weighted (by selectivity) sum over ages of population weights-at-age. Population weight-at-age was simply the product of abundance-at-age $N_{a,y}$ and expected weight-at-age of an individual (w_a, assumed known as described above). Thus, the survey CPUE index in year y was

$$(7)\quad C_{s,y} = q\Sigma_a\, w_a\, s_{s,a}\, N_{a,y}\,.$$

The proportionality constant q was determined by a constraint as described below.

Generation of Simulated Data

For each run, a new simulated data set was generated. All parameters defining the simulation model are given in Table 1, along with their values or the distribution from which they were generated.

The simulation model made essentially the same assumptions as were incorporated into the stock synthesis model described above. Given the initial age composition, recruitments, fishing mortality rates, and other (fixed) parameters (Table 1), we used equations 1–5 to simulate population dynamics and fishery catch over time. M was fixed at 0.15, and the same set of fishery selectivity parameters (Table 1) was used in all cases, leading to a dome-shaped selectivity function (Fig. 1).

Each simulation run used randomly generated recruitments ($N_{5,y}$, $y = 1, \ldots, 15$), initial numbers at age ($N_{a,1}$, $a = 6, \ldots, 13$), and fishing mortalities (F_y, $y = 1, \ldots, 15$) in each year. Recruitment was generated from a lognormal distribution with specified mean and variance (Table 1, estimated from Hightower and Lenarz 1989). Initial numbers-at-age were calculated from randomly generated recruitment values, reduced for the cumulative natural mortality up to the age in question. As indicated above, this assumes insignificant fishing prior to the first year of the simulation. For age 13+, initial numbers were generated and summed for the youngest 50 year-classes in this age-group (numbers at older ages were negligible). Fishing mortality rate on age 10 fish (F_y), with the mean appropriate for the given set of runs, was generated from a lognormal distribution. The CV for F_y was 30%, roughly approximating the variability in estimated fishing mortality seen in a number of different assessments of rockfish stocks (PFMC 1990). Note that the expected fishing mortality rate was constant over years and was not changed in response to the current estimate of the status of the stock.

In this model the expected level of recruitment was not a function of the biomass of the stock when the year-class was generated, and this implies that substantial compensation was taking place. The assumption was useful because direct effects of fishing on adult stock composition were not confounded with indirect effects on recruitment that would cause the average magnitude of recruitment to differ between low and high fishing mortality rates.

Both the fishery and survey age-composition data for each year were generated as multinomial samples with effective sizes of 400. The expected proportions-at-age were calculated directly from catch-at-age (equation 5) or by equation 6 (the survey). Some estimates of the variance of catch-at-age numbers for west coast groundfish suggest that our admittedly somewhat arbitrary choice of effective sample size (after Fournier and Archibald 1982) may not be unrealistic (Sen 1986; Methot 1990; Parker and MacCall, unpublished manuscript[2]). The total biomass caught by the fishery was assumed to be measured precisely, so observed catches were generated from a lognormal distribution with a CV of 1% about the true value, matching the error assumed by the assessment methodology in likelihood calculations (see below). The survey index of biomass was generated from a lognormal distribution with CV's of either 10, 25 or 50%, depending upon the particular run, and an expected value specific to the given year of the run. The expected index value was the sum of the products of expected survey catch-at-age and weight-at-age. The expected survey catch-at-age is given by numerator of equation 6, multiplied by q (which was fixed at 1.0 for simplicity). We use the same weight-at-age relationship here as was assumed above in the synthesis model.

[2]K.R. Parker and A.D. MacCall. 1990. An interactive port sampling model for California groundfish. Administrative Report T-90-03, Tiburon Laboratory, 3150 Paradise Drive, Tiburon, CA.

Fitting the Synthesis Model to the Simulated Data Sets

For each run the numbers in age categories 6–13+ in the initial year ($N_{6,1}$–$N_{13,1}$), recruitment ($N_{5,1}$–$N_{5,15}$) in each year, four selectivity parameters for the fishery and four for the survey, F_y in each year, and q (the proportionality constant for the survey) were estimated. Of the above, the F_y's and q were determined by constraints, and the others are estimated as formal parameters. Other values used in generating synthetic data and calculating likelihoods either were constants assumed known a priori or were calculated from the formally estimated parameters, parameters determined by constraints, and the assumed constants.

As mentioned above, our estimates of F_1–F_{15} and q were obtained by requiring them to satisfy constraints. The F_y's were continually updated along with the formal parameters so that the estimated catch biomass in each year exactly matched the observed catch, if this were possible (Methot 1990). A likelihood component for the catch biomass was included in the fitting so that solutions that did not allow the catch biomass to be matched (e.g., if an F is fixed so that the population cannot support the observed catch) were penalized for this lack of fit through smaller likelihoods. Because the survey is a relative one, the survey proportionality constant, q, is chosen so that the sum of the model's estimated survey catches over years equals the observed sum based on the data (Methot 1990).

The set of formal parameter estimates and constrained values that maximized the log-likelihood function was chosen as the best fit (Fournier and Archibald 1982; Kimura 1990; Methot 1990). The log-likelihood (L) was

$$(8) \quad L = L_1 + L_2 + L_3 + L_4$$

where L_1 was the log-likelihood associated with the fit to the fishery age composition, L_2 was the log-likelihood associated with the fit to the survey age-composition data, L_3 was the log-likelihood associated with the biomass of fishery catch, and L_4 was the log-likelihood associated with the survey index of biomass. These individual components (ignoring constant terms) were

$$L_1 = \Sigma_y\, 400\, (\Sigma_a\, \tilde{P}_{f,a,y} \log \hat{P}_{f,a,y})$$

$$L_2 = \Sigma_y\, 400\, (\Sigma_a\, \tilde{P}_{s,a,y} \log \hat{P}_{s,a,y})$$

$$(9) \quad L_3 = \Sigma_y - 0.5 \left\{ \frac{\log\left(\frac{\hat{C}_{f,y}}{\tilde{C}_{f,y}}\right)}{\sigma_f} \right\}^2$$

$$L_4 = \Sigma_y - 0.5 \left\{ \frac{\log\left(\frac{\hat{C}_{s,y}}{\tilde{C}_{s,y}}\right)}{\sigma_s} \right\}^2$$

where a tilde indicates observed data and a circumflex indicates values estimated by the stock synthesis model, $P_{f,a,y}$ represents

the proportion of the sample type t (either fishery (f) or survey (s) in year y that is age a, and σ_t indicates the (assumed known) standard deviation on a log-scale of $\tilde{C}^t_{y,a}$.

L_1 and L_2, the log-likelihood components for the fishery and survey age compositions, reflect the assumption that the observed proportions-at-age for each year were produced as though the sampled fishery catch or sampled survey catch were multinomial samples of size 400. This "effective sample size" of 400 is internal to the likelihood component and acts to weight the magnitude of the component based upon the assumed precision associated with the sampling program. The value of 400 used here matched the actual sample size used to generate the age-composition data (see above). In practice, an analyst could estimate the effective sample size for each year based on independent information on the precision of the catch sampling program (Methot 1990). Our approach assumed that the effective sample size was constant, and more importantly, that it had been estimated correctly.

The likelihood components for the fishery catch biomass and the survey index were of lognormal form. Thus, the likelihood increased as the squared errors between the log-transformed observed and synthetic values decreased. The values of σ_f and σ_s were assumed known and act to weight the two components in the total log-likelihood. They were determined by the assumed level of error associated with each type of data. σ_f was set at a value corresponding to a CV of 1% on the untransformed scale, which matches the level of error used to produce the observed (simulated) data. σ_s was set at values corresponding to CV's of 10, 25, or 50% on the untransformed scale, with the level of error chosen to match the level of error used to produce the observed (simulated) data for the given run.

For interested readers, the next two subsections provide technical detail on the process of searching for the maximum likelihood solution.

Numerical Details of the Fitting Procedure

The maximum likelihood solution was found using a Broyden–Fletcher–Goldfarv–Shanno update procedure combined with a cutback line search, a standard quasi-Newton method (Gill et al. 1981). Convergence was assumed when both the maximum change in any formal parameter value was less than 0.1% and the total log-likelihood changed less than 0.001%. Initial tests indicated that more stringent convergence criteria did not lead to improved estimates.

Our experience in fitting age-structured models to fishery data has indicated that results can be sensitive to initial parameter values, so we attempted to mimic the approach an analyst might use to specify initial values. Initial numbers at age and recruitment in each year were set by doing a cohort analysis (Pope 1972), following the approach used in Deriso et al.'s (1985) catch-at-age software (CAGEAN). Initial values for parameters determining fishery selectivity were set as follows. First, the $F_{a,y}$'s estimated by the cohort analysis were divided by $F_{y,10}$. These were then averaged over years to obtain an estimate of $s_{f,a}$ for each age, and the double-logistic function was fit to these estimates by least squares. The shape of the survey selectivity function was assumed to be essentially unknown, and a single set of initial values that produced a mildly dome-shaped selectivity function was used in all runs.

Local versus Global Maxima on the Likelihood Surface

When maximizing a parameter-rich function, there is a real danger that a solution may only maximize the likelihood function locally. This is of special concern in simulation studies because it is generally not practical to simulate the critical review of a stock assessment scientist for each individual run. We found that the model occasionally converged to solutions that did not have the global maximum likelihood. We discovered this by first noting that in some cases the estimated fishery selectivity function was asymptotic, or more rarely flat or decreasing, instead of the correct dome-shape. In a separate set of runs, we examined whether these solutions were true maximum likelihood solutions by fixing the fishery selectivity parameters at the correct values and reestimated the remaining parameters. Cases that originally estimated either a flat or declining fishery selectivity curve showed a substantial increase in the likelihood function, while likelihoods were similar to original values for cases where the original estimated fishery selectivity was either dome-shaped or asymptotic. In some test cases, we did not have success reaching the true maximum likelihood solution by restarting the fitting process from the incorrect solution.

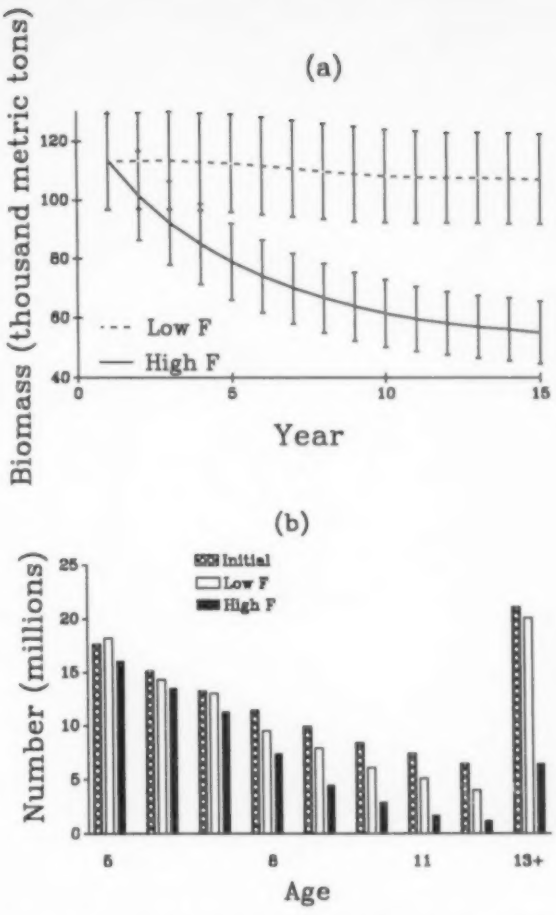

FIG. 2. True population dynamics of simulated populations. (a) Average biomass (± SD) plotted against time for high and low fishing mortality rates and (b) average initial and final numbers-at-age for low and high fishing mortality rates.

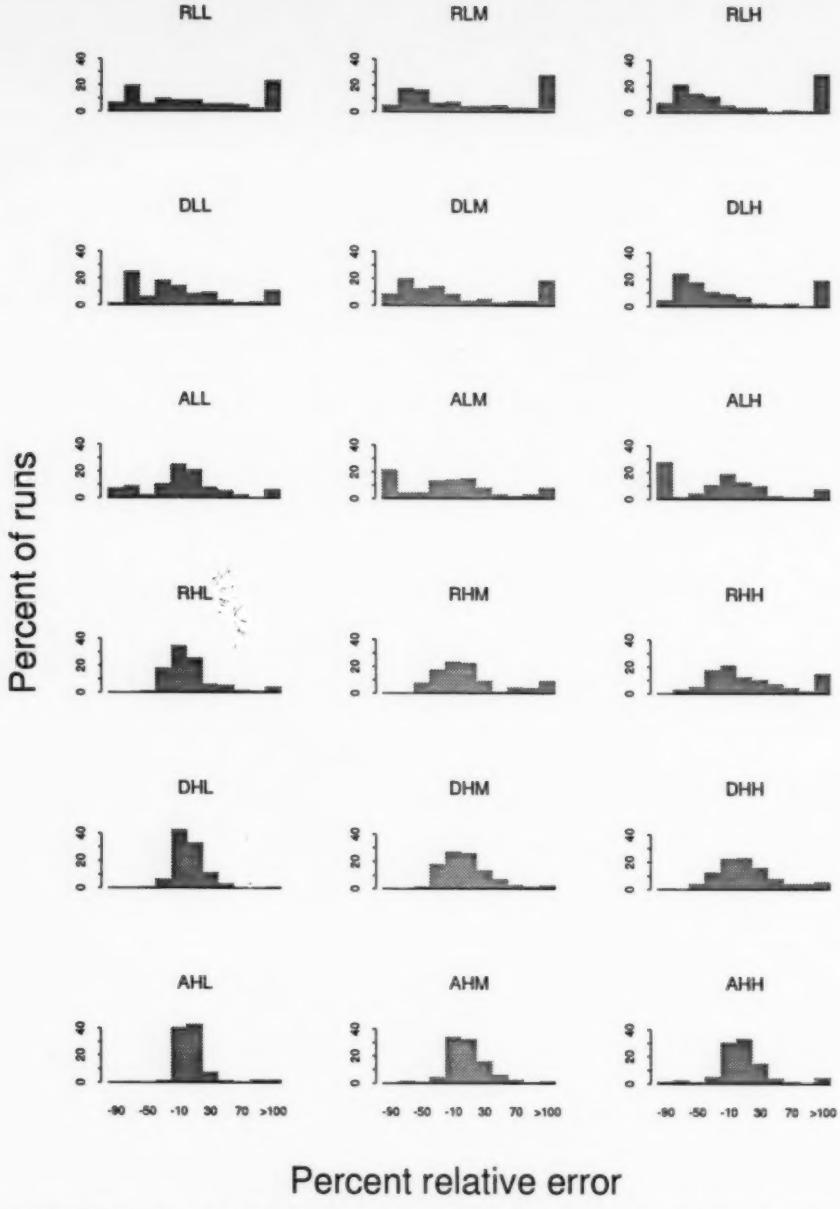

FIG. 3. Distributions of relative errors in estimated year 15 biomass for each case. Headings of the form "XYZ" denote the survey selectivity (X = R, D, or A for recruit, dome-shaped, or asymptotic, respectively), the fishing mortality rate (Y = L for low ($0.075 \cdot yr^{-1}$) or Y = H for high ($0.3 \cdot yr^{-1}$), and the survey coefficient of variation (Z = L, M, or H for low (10%), medium (25%), or high (50%), respectively) for each case.

In real assessments, we expect that declining or flat selectivity curves would be considered suspect, and additional runs would be done with different initial values for the parameters, so that a solution close to the maximum likelihood might be found. A real analyst might also run the stock synthesis model with a grid of starting values and potentially avoid the problem we encountered in the cases described above. We have therefore excluded runs that, by inspection, would be categorized as nearly flat or generally declining. (We defined as nearly flat or declining any case where the estimated fishery selectivity for age- 5 fish was greater than 70% of the maximum estimated selectivity; this is substantially above the true selectivity at age 5, which is 17% of the maximum, and nearly all values over 25% were also over 70%.) Because of the rarity of the "flat" and declining estimated curves (about 5% of the runs), excluding these runs does not influence any of our qualitative con-

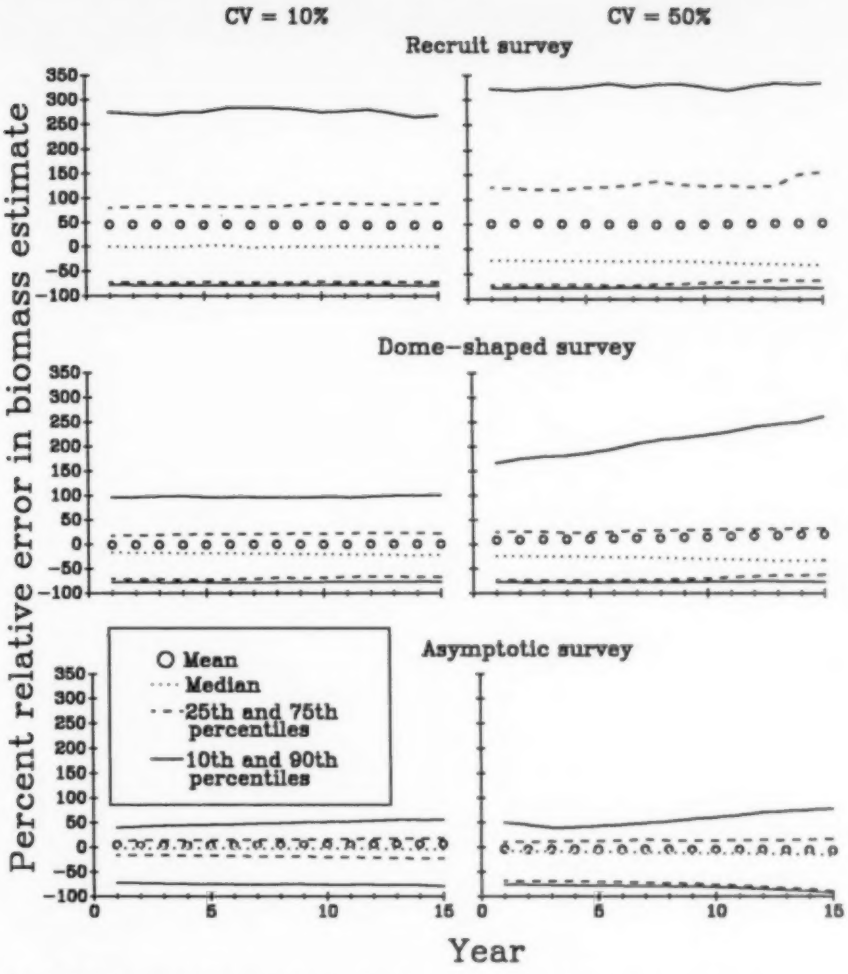

FIG. 4. Relative error in biomass estimates plotted aginst year for the low fishing mortality rate. Shown are results for survey CV's of 10 and 50%. For conciseness, results for survey CV's of 25% are not displayed; they were intermediate between the displayed cases.

TABLE 2. Percentage of runs with estimates of year 15 biomass within 20% of the correct value.

Survey type	Fishing mortality	Survey variability		
		Low	Medium	High
Recruit	Low	16.8	11.8	8.8
	High	60.6	46.1	33.7
Dome-shaped	Low	22.6	12.0	16.2
	High	76.0	53.0	45.5
Asymptotic	Low	46.2	29.2	32.0
	High	84.2	66.5	64.0

clusions. If included, these runs would accentuate the patterns presented below because the cases with the most variable results also produced more estimated flat and declining selectivity functions, and runs with these erroneous selectivity patterns also tended to have large errors in other parameters.

Results

Population Dynamics

Under the high fishing rate scenario, average biomass fell by about half from year 1 to 15, and the contribution of the older ages, particularly the 13+ category, declined markedly (Fig. 2). On average, biomass leveled off in the final years of the simulations because the population was coming into balance with the higher mortality imposed by fishing. Under the lower fishing rate, the decline in biomass was only slight, and the shift in the age distribution was not marked.

Skewed Distribution of Errors

We examined the distributions of percent relative error (PRE) = 100 × (estimated-true)/true for each run both for biomass and recruitment estimates. For each type of survey the distribution of errors was skewed, so that the mean PRE typically exceeded the median (Fig. 3–7). Median errors were typically

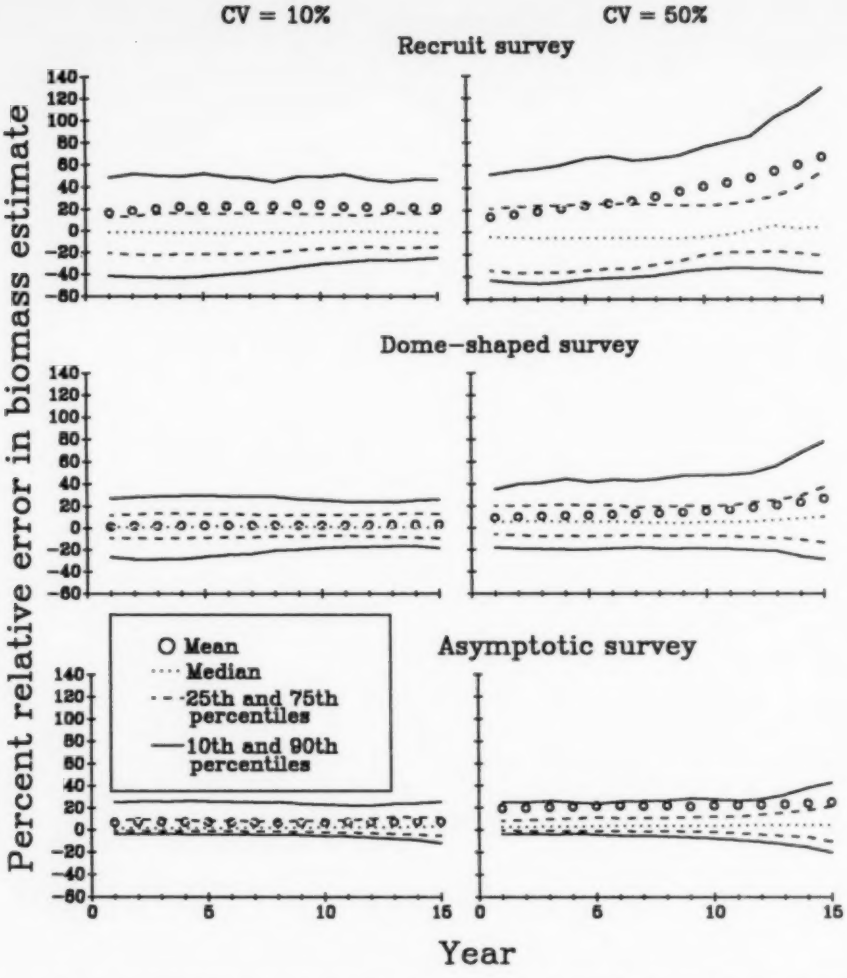

FIG. 5. Relative error in biomass estimates plotted against year for the high fishing mortality rate. Shown are results for survey CV's of 10 and 50%. For conciseness, results for survey CV's of 25% are not displayed; they were intermediate between the displayed cases.

close to zero. Given the variability in the magnitude of errors and the skew of the distributions, it was clear that the mean error alone would sometimes be an insufficient measure of the performance of the stock synthesis model.

Accuracy and Precision in Biomass and Recruitment Estimates

For management purposes, the accuracy of biomass estimates in the final year of an assessment is usually of greatest interest. Consequently, we examined the distribution of relative errors in estimated biomass in the 15th year (Fig. 3) and also tabulated the percentage of runs with year 15 biomass estimates within 20% of the correct value (Table 2). Although 20% is a somewhat arbitrary cutoff, we felt that stock assessments with errors less than 20% would be classified as successful by most workers. The overall pattern in the results is for greater accuracy and precision given a higher fishing mortality level and when survey precision was higher (Table 2; Fig. 3). Errors were most concentrated around zero for the asymptotic survey and least concentrated around zero for the recruit survey (Table 2; Fig. 3). The difference in accuracy and precision between the recruit and asymptotic surveys was comparable with differences within a survey type when its CV ranged from 10 to 50%. The difference between the asymptotic and dome-shaped surveys was accentuated at a low fishing rate (Table 2; Fig. 3). This latter result is reasonable because the asymptotic survey sampled older fish to a greater extent, and these constituted a larger proportion of the year 15 stock at a low fishing rate (Fig. 2).

Estimates of biomass and recruitment in earlier years are also of interest because they can provide information on how the stock is changing over time. In general, the precision and accuracy of biomass (Fig. 4 and 5) and recruitment (Fig. 6 and 7) estimates over the entire time series showed the same qualitative patterns as year 15 biomass estimates. The most accurate and precise estimates came from the asymptotic survey and the least accurate and precise from the recruit survey. As might be

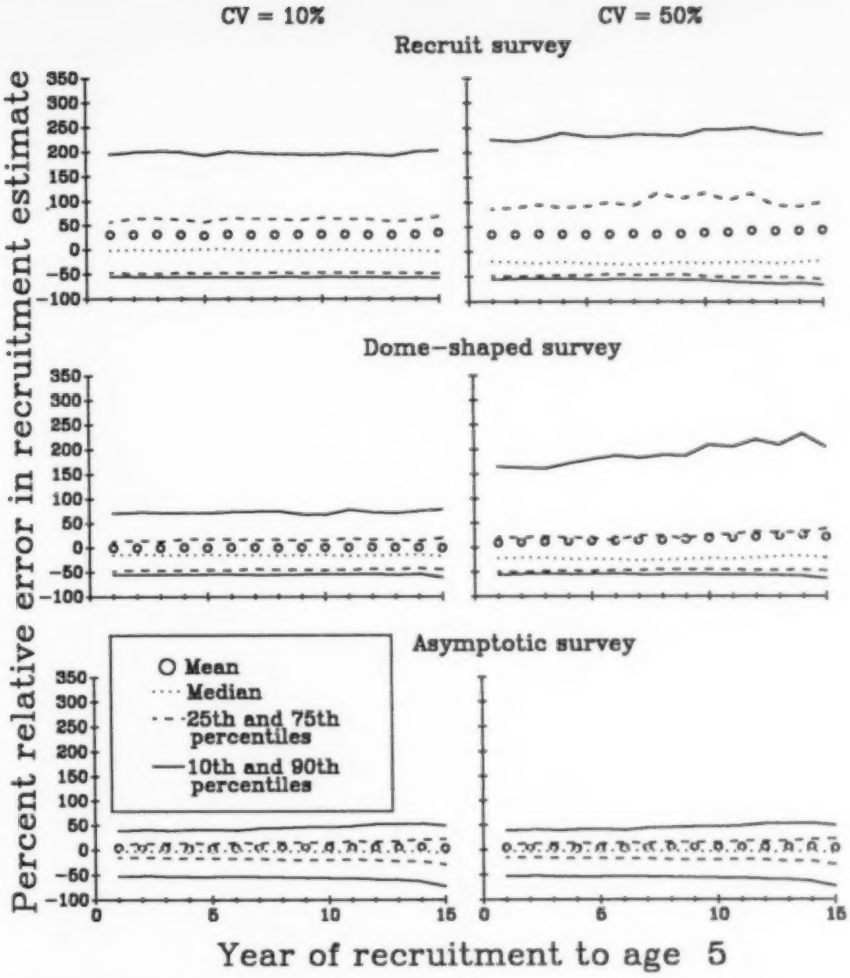

FIG. 6. Relative error in recruitment estimates plotted against year for the low fishing mortality rate. Shown are results for survey CV's of 10 and 50%. For conciseness, results for survey CV's of 25% are not displayed; they were intermediate between the displayed cases.

expected, estimates were better when the survey CV was lower or the fishing mortality rate was higher.

Errors in estimated biomass in year 15 were generally associated with an overall scaling error. These scaling errors caused the correlation between errors in biomass estimates and errors in initial number and recruitment parameter estimates to be high, although the correlations did fall off some at the beginning and end of the time series (Fig. 8). The correlation between errors in recruitment (or initial numbers-at-age) and errors in year 15 biomass estimates tended to be weakest in cases where absolute estimates of biomass were best. For example, correlations were generally lower at the higher fishing mortality rate, especially for the dome-shaped and asymptotic surveys with a CV of 10%.

To the extent that errors were due to an overall scaling problem, estimates of relative year-class strength might still be quite good even when absolute estimates were poor. We measured relative recruitment, on a log scale, as log-transformed recruitment minus the mean of log-transformed recruitment. Estimated and true values of relative recruitment were highly correlated (Fig. 9). Although lower, the correlations were still substantial even at the end of the time series. The lowest correlations were for the dome-shaped survey at the end of the time series of recruitment estimates. This is not unexpected because these year-classes were never well sampled by this survey.

Substantial errors in year 15 biomass were sometimes associated with trends in estimated recruitment, as estimated by linear regression of recruitment estimates against year. Stong trends were most evident when the fishing mortality rate was low, especially when final biomass was substantially overestimated (Fig. 10). For this case, although more trends were positive, some strong trends were negative, and in these cases, biomass in the first year was overestimated to an even greater degree than year 15 biomass. Strong trends were less common at the higher fishing mortality rate, and underestimates tended to have more declining trends and overestimates more increasing trends (Fig. 10).

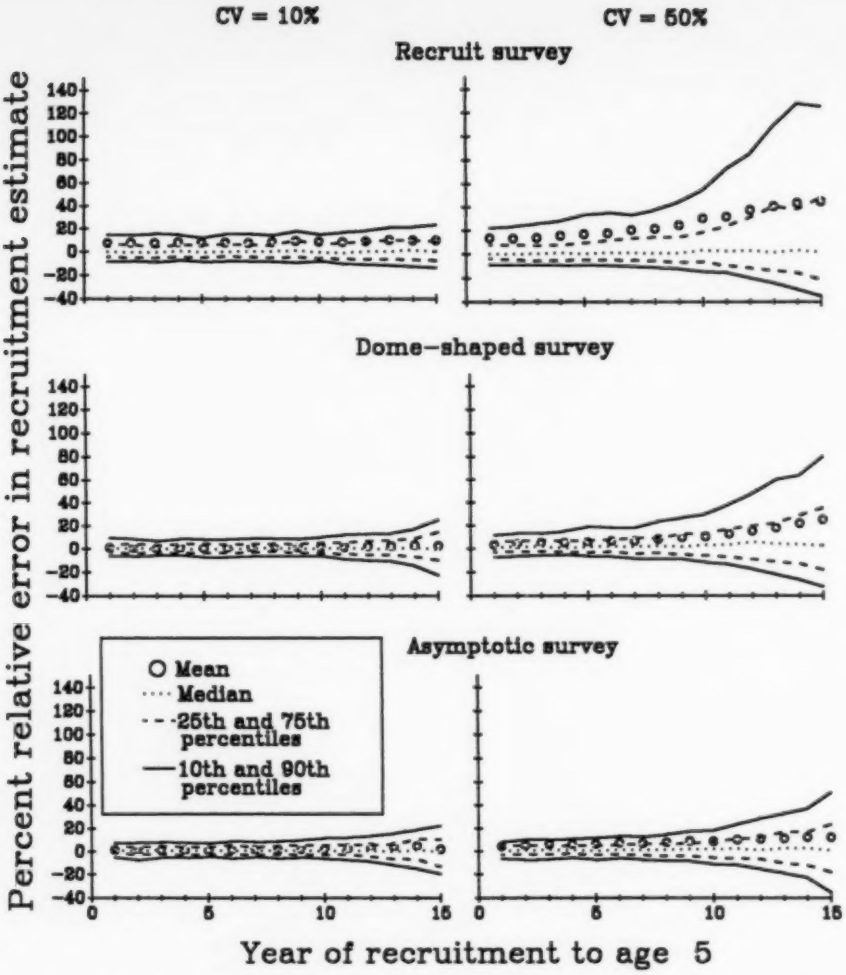

FIG. 7. Relative error in recruitment estimates plotted against year for the high fishing mortality rate. Shown are results for survey CV's of 10 and 50%. For conciseness, results for survey CV's of 25% are not displayed; they were intermediate between the displayed cases.

Temporal Patterns in Biomass and Recruitment Estimates

By analogy with cohort analysis, it is reasonable to expect convergence of estimated values of recruitment as we move back in time, with the convergence being stronger at higher fishing mortality rates (Pope 1972). To the extent that the convergence property carries over to separable models, and the convergence is toward the true value, we would expect to see tighter distributions of relative errors in estimated recruitment earlier in the time series and at higher fishing mortality rates. Our results match this expectation. At the lower level of fishing mortality, there appears to have been only slight convergence in earlier years toward the correct value of recruitment (Fig. 6). In contrast with the low fishing rate results, there was substantial convergence for estimates of recruitment at the high level of fishing mortality (Fig. 7).

In a number of cases, biomass estimates improved as we moved backward in time (Fig. 4 and 5). This, however, is not a general result. For example, at a high fishing mortality level with a low-variability dome-shaped survey, the most accurate biomass estimates are in year 10, with slightly less accurate estimates in earlier years (Fig. 5). This may have occurred because some of the cohorts contributing to biomass in the early years were observed only for a few years before moving into the 13+ category (see also Kimura 1989).

Discussion

In this paper, we used Monte Carlo simulation to evaluate the performace of the stock synthesis model. Our study was unique in its assessment of the value of a relative survey (i.e., one proportional to available stock, but with the proportionality constant unknown) to the synthesis model, and especially in the comparison of such surveys with different selectivity patterns. Although we only examined the stock synthesis model in our simulations, we suspect that many of our results apply more generally to statistically based catch-at-age methods such as

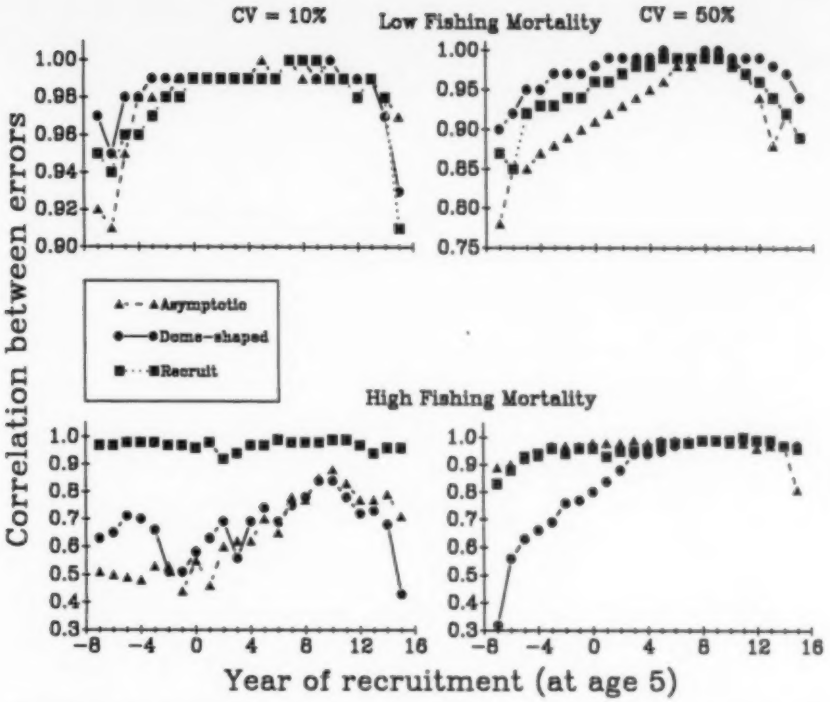

FIG. 8. Correlation between relative error in estimated biomass in year 15 and relative error in estimates of initial numbers-at-age and recruitment parameters. Correlation is plotted against the year of recruitment to age 5 associated with the parameter. Thus, the correlations with recruitment parameters ($N_{5,y}$, $y = 1, \ldots, 15$) are plotted at X values of $y = 1, \ldots, 15$, and the correlations with initial numbers-at-age ($N_{a,1}$, $a = 6, \ldots, 13$) are plotted at X values of $a - 6 = 0, -1, \ldots, -7$. For example, the correlation between the relative error in estimated year 15 biomass and initial numbers at age 8 ($N_{8,1}$) is plotted at an X value of -2 because age 8 fish in year 1 recruited at age 5 3 yr earlier. For conciseness, results for survey CV's of 25% are not displayed; they were similar to displayed results.

CAGEAN (Deriso et al. 1985) and perhaps also to tuned virtual population analysis (e.g., Pope and Shepherd 1985). Care should be taken in extrapolating from our results to short-lived species because the role of survey selectivity could be quite different when the population is composed of only a few age-classes.

Although tangential to the main thrust of this paper, we think that it is of interest that the synthesis estimates of recruitment and biomass were generally positively biased. In contrast with the mean errors, median errors were generally quite close to zero. This kind of "transformation bias" is a well-known phenomenon arising from the skew in the lognormal distribution of errors we assumed. We suspect that such a bias is built into many catch-at-age methods and is probably a real phenomenon because in practice, most distributions of errors will be skewed. However, whether a median or mean error of zero (or something else) is desired depends in part upon management goals (see also Pope and Shepherd 1985).

In our study, an asymptotic survey was best for estimating current stock size within some fixed percentage of the correct value. Accuracy fell some for the dome-shaped survey, and the recruit survey produced estimates that were considerably less accurate than those associated with the other survey types. These results suggest that, all else being equal, an asymptotic survey should be chosen. The extent, however, to which the selectivity pattern of a survey can be controlled or chosen is of pragmatic concern. For example, fish behavior, distribution, and ability to avoid capture may make it difficult to design an asymptotic survey. However, a combination of surveys that together capture a broad range of ages may provide many of the benefits we have associated with an asymptotic survey. In the end, choices among survey types will involve their relative costs, the value of forecasting future recruitment (e.g., Walters 1989), as well as the expected accuracy of the resulting estimates of current biomass. When these trade-offs are being made, it is important to keep in mind that differences in the performance of surveys with different selectivity patterns can be quite substantial, rivaling differences in performance resulting from severalfold differences in coefficients of variation in survey error.

A relative survey can be used to help determine absolute abundance primarily because it provides information on how the fished stock changes from year to year. The magnitude of these relative changes can then be related to the absolute magnitude of the fishery catch. The poor performance associated with the recruit survey can be understood by noting that most fish captured by the recruit survey have not yet experienced fishing mortality. Thus, changes in survey CPUE reflect year-to-year variations in relative recruitment, but are largely uncoupled from changes in the stock due to fishing. Furthermore, the

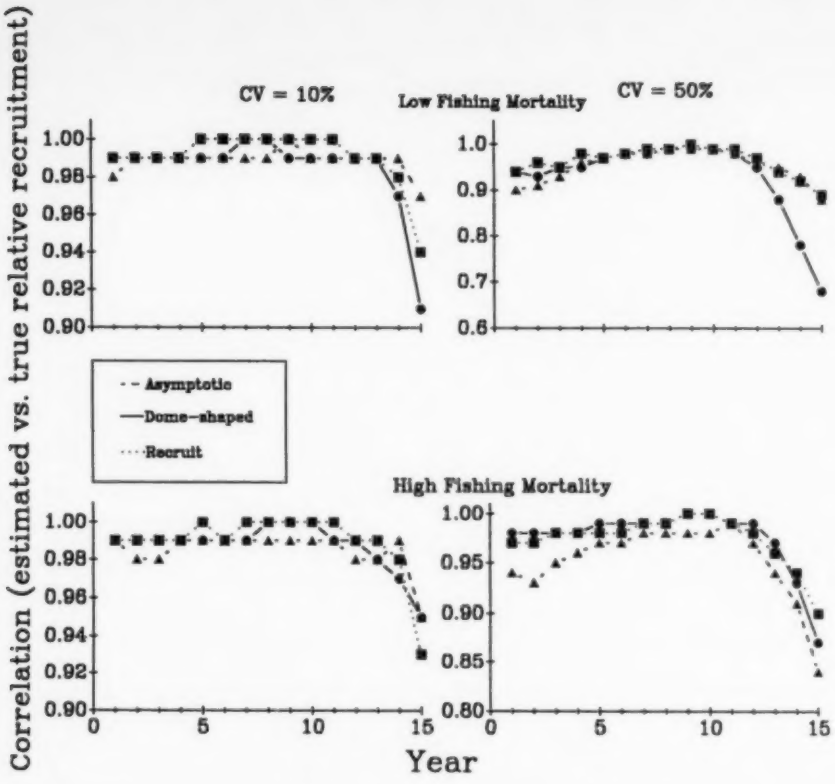

FIG. 9. Correlation between estimated relative recruitment and actual relative recruitment plotted against the year of recruitment. For conciseness, results for survey CV's of 25% are not displayed; they were similar to displayed results.

fishery age-composition data alone already contain substantial information on relative year-class strength (e.g., Ricker 1975, p. 265–272), but essentially none on absolute stock size (Doubleday 1976; Deriso et al. 1985; Shepherd and Nicholson 1986). Consequently, much of the information on relative year-class strength provided by the recruit survey is redundant and does not provide information on absolute abundance. Indeed, for the recruit survey, our stock synthesis estimates of relative recruitment were quite good, even though absolute estimates of recruitment and biomass tended to have large errors.

When fishing mortality has been low, assessments of current stock size based on fishery catch-at-age data and a survey of relative abundance will often be substantially in error. Under higher cumulative fishing mortality, it is intuitive that catch-at-age analyses give better results because such a large proportion of each cohort of fish are counted in the catches (Pope 1972). However, our results suggest that even when F is high, the relative survey must be highly precise to have a high probability that an estimate of current abundance will be within 20% of the correct value. Although large errors in estimated year 15 biomass were sometimes associated with incorrect patterns for the fishery selectivity function in our study, most of the estimated patterns were not "pathological," and we doubt that an analyst could rule them out as unreasonable without making use of information external to the assessment. With a very long time series, a relative survey might fix absolute abundance adequately, even at a low fishing mortality rate, but then the assumptions of constant natural mortality, selectivity patterns, growth, etc., are probably untenable (e.g., Walters 1986).

We believe that our simulation study presents a best-case scenario for stock assessments. In the simulations, selectivity or weight-at-age did not change over time, natural mortality was assumed known and remained constant (over years and ages) at the assumed value, and catch biomass was assumed to be known accurately. The precision of our simulated survey varied from high (CV = 10%) to modest (CV = 50%), and we assumed that there was a survey every year. In practice, the real population will deviate from the synthesis (or other) model to an unknown extent, and the quality and quantity of data will often be lower than in our simulations.

To place our simulations in context, it is worth considering the actual kinds of data usually available for use with the stock synthesis model. Assessments of several groundfish stocks on the west coast of North America make use of trawl survey data collected every third year with CV's near 50% (Bence and Rogers 1992; Rogers and Bence 1992). For many stocks, natural mortality was estimated by catch curves, maximum ages, or simply by analogy with other species (e.g., Hightower and Lenarz 1986; Bence and Rogers 1992; Rogers and Bence 1992). In our simulations, we generated data with a very low level of sampling error for catch biomass because this is the assumption under which the synthesis model is fit to the data. However, we recognize that catch biomass estimates are probably less accurate than the low level of error built into our simulations

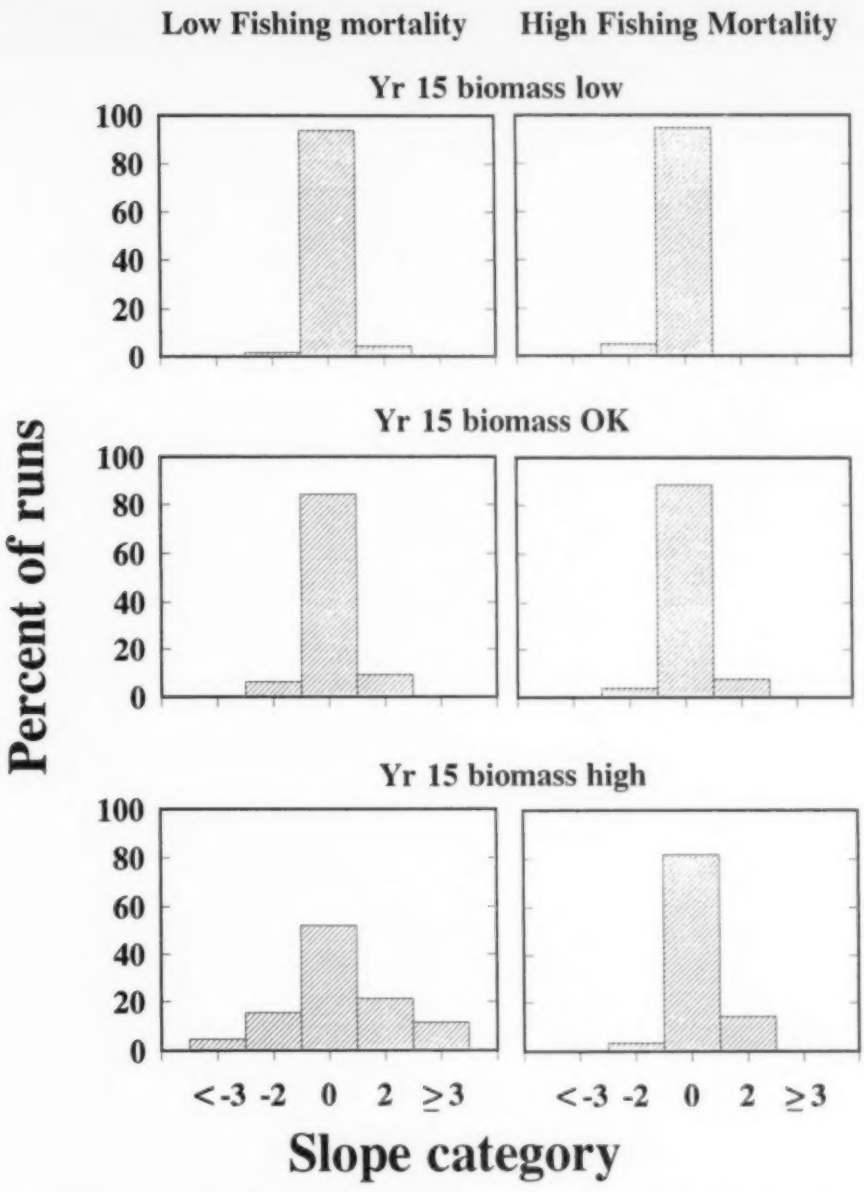

FIG. 10. Distribution of estimated slopes (by linear regression) of the trend in estimated recruitment over time. Distributions were calculated separately for replicates with low (more than 20% low), OK (within 20% of the correct value), or high (more than 20% high) estimates of year 15 biomass. Shown are results for an example low and high fishing mortality case (dome-shaped selectivity, CV = 25%); other cases showed similar patterns.

because of two general problems. First, discard levels generally are estimated roughly from an observer program of restricted spatial and temporal extent. Thus, even when landings are made in nearly pure "market categories" and reported landings are essentially a census of the landed catch, as they are for widow rockfish, the error in catch biomass could be substantial. Second, estimation of landed catch is complicated for some stocks because landings are made in mixed-species market categories that need to be sampled (Bence and Rogers 1992). This can sometimes cause the estimates of landings to have substantial sampling error. For most assessments using the synthesis model, age-composition data appear to be quite good, at least as representations of the landed catch. Estimated proportions at age in the landed catch often come from extensive sampling at the ports (stratified both spatially and by time of year) combined with the reading of thousands of otoliths.

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Influence of Overwintering Stresses on Respiration Throughout the Life History of the Freshwater Leech, *Nephelopsis obscura*

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Davies, R.W., and V. Kalarani. 1993. Influence of overwintering stresses on respiration throughout the life history of the freshwater leech, *Nephelopsis obscura*. Can. J. Fish. Aquat. Sci. 50: 841–845.

The effects of overwintering stresses (low temperature, low oxygen concentration) on the life history patterns of activity-specific oxygen consumption by *Nephelopsis obscura* were determined using a flow-through respirometer system and compared with leeches hatched and maintained under summer conditions. While resting and active oxygen consumption increased with body dry weight, weight-specific resting (Rm) and active (Ra) oxygen consumption and aerobic scope (AS) decreased with increase in body weight in both winter and summer *N. obscura*. Rm in winter leeches was higher than in summer leeches, probably reflecting the higher metabolic costs of tissue repair and maintenance after winter stresses. Although Ra and AS in winter leeches were initially lower than in summer leeches, by stage 4, compensation in winter leeches was complete and by stage 6, over-compensation occurred. The effects of overwintering on oxygen consumption and AS persisted throughout the life history and help explain some of the differences in allocation of energy storage observed in winter and summer leeches.

Les effets des stress de l'hiver (faible température, faible concentration d'oxygène) sur l'évolution de la consommation d'oxygène spécifique de l'activité au cours du cycle vital de *Nephelopsis obscura* ont été déterminés à l'aide d'une système respirométrique à renouvellement de l'eau et les résultats ont été comparés aux valeurs obtenues chez des sangsues écloses et gardées dans des conditions caractéristiques de l'été. Bien que la consommation d'oxygène au repos et en activité ait augmenté avec le poids corporel sec, la consommation d'oxygène au repos (Cr) et en activité (Ca) spécifiques du poids et l'étendue aérobie (EA) ont diminué avec l'augmentation du poids corporel chez *N. obscura* aussi bien l'hiver que l'été. La Cr était plus élevée chez les sangsues l'hiver que l'été, ce qui reflète probablement les coûts métaboliques plus élevés liés à la réparation et à l'entretien des tissus après les stress de l'hiver. Bien que la Ca et l'EA aient été initialement plus faibles chez les sangsues d'hiver que chez les sangsues d'été, la compensation chez les sangsues d'hiver était complète au stade 4, et au stade 6, il y avait surcompensation. Les effets de l'hiver sur la consommation d'oxygène et sur l'EA ont persisté pendant toute la durée du cycle vital et aident à expliquer certaines différences dans l'affectation de l'énergie à la croissance somatique et au stockage d'énergie observées chez les sangsues d'hiver et d'été.

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The influence of body size on oxygen consumption has been well documented for numerous freshwater poikilotherms and leeches (Mann 1956; Ramamurthi 1965; Kulkarni 1978). The effects of temperature, oxygen saturation, osmotic stress, and food deprivation on oxygen consumption have also been studied in a diversity of freshwater invertebrates (e.g., Rockwood et al. 1990; Urabe and Watanabe 1990; Glazier 1991; Hornbach 1991), including leeches (Lindeman 1932, 1935; Mann 1956, 1958, 1961; Madanmohanrao 1960; Ramamurthi 1965, 1968; Calow and Riley 1982; Wrona and Davies 1984; Milne and Calow 1990; Davies et al. 1992). Closed respirometers have been used most frequently in these studies despite their many associated problems (Lampert 1984; Wrona and Davies 1984). Flow-through systems overcome these problems and allow accurate quantification of the activity patterns of an organism concurrent with the measurement of oxygen consumption (Wrona and Davies 1984; Davies et al. 1992).

Nephelopsis obscura Verrill (Erpobdellidae) is a predatory leech widely distributed in lentic freshwater ecosystems of Canada and the northern parts of the United States (Davies 1991). In southern Alberta, two generations of *N. obscura* are produced annually, one in early summer and the second in late summer, both generations overwintering prior to reaching maturity the following summer. Each generation produces young after either 12 and 15 or 12 and 19 mo. Before entering winter, *N. obscura* of the early summer generation are >90 mg and those from the late summer generation are between 9 and 19 mg (Davies and Everett 1977). Thus, 3- to 5-mg individuals collected at the end of spring before reproduction commences belong to the late summer generation which overwinters when the lakes are anoxic or severely hypoxic for 90–120 d (Baird et al. 1987) and when, like the majority of freshwater invertebrates in temperate zones, it also experiences low water temperatures and restricted food availability. Dratnal and Davies (1990) and Reddy et al. (1992) showed that small overwintered *N. obscura* and similar sized individuals hatched in the summer differed not only in age but also in other ecophysiological processes (although respiration was not measured) resulting in differences in life history and success. The physiological differences between winter and summer *N. obscura* persisted

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TABLE 1. Mean (± SD) WWT and DWT (mg) of winter and summer *N. obscura* ($n = 5$) at each of the six life history stages.

Leech group	Stage 1	2	3	4	5	6
Winter						
WWT	9.9 ± 3.0	21.9 ± 3.1	165.4 ± 17.9	279.1 ± 29.1	349.3 ± 28.6	515.3 ± 74.5
DWT	1.8 ± 0.4	4.1 ± 0.5	30.8 ± 1.8	56.7 ± 2.7	77.2 ± 3.4	107.5 ± 4.6
Summer						
WWT	11.1 ± 2.4	23.1 ± 2.0	163.1 ± 9.4	297.4 ± 12.2	478.7 ± 31.5	704.2 ± 58.7
DWT	2.0 ± 0.3	4.5 ± 1.1	31.5 ± 1.8	60.8 ± 5.4	86.4 ± 6.3	145.6 ± 11.5

throughout the life history. Winter *N. obscura* started the summer season with significantly lower total lipid, total protein, and glycogen concentrations than similar sized summer leeches. Summer leeches allocated energy to storage approximately isometrically with an increase in body size. In contrast, winter leeches compensated for winter stress by enhanced energy allocation to total lipid, total protein, and glycogen during the early life history stages.

This is the first study to determine the effects of a stress (in this case, overwintering) on activity-related oxygen consumption (a measure of respiration) throughout the life history of a species (*N. obscura*). Specifically, we determined if exposure to winter stresses results in changes in resting oxygen consumption, active oxygen consumption, and aerobic scope compared with summer (control) leeches and whether compensation occurs in winter leeches.

Materials and Methods

Small (8–12 mg wet weight (WWT)) *N. obscura* that had overwintered in the field (termed winter *N. obscura*) were collected from Stephenson's Pond near Calgary, Alberta, during May 1990 when the water temperature was 4°C. They were gradually acclimated to 20°C ($1°\cdot d^{-1}$) with 100% oxygen-saturated filtered pond water and a 12 h light: 12 h dark regime and provided ad libitum with *Tubifex tubifex* Müller for 2 h three times a week until they reached maturity. In July 1990, *N. obscura* cocoons were collected form the pond immediately after deposition (water temperature 20°C) and maintained in the laboratory in filtered aerated pond water at 20°C with 100% oxygen saturation for approximately 4 wk until they hatched (termed summer *N. obscura*). After hatching, they were maintained under identical conditions to the winter *N. obscura* until they reached maturity.

Both winter and summer *N. obscura* showed sigmoid growth, and six representative life history stages were selected (Table 1), each characterized by differences in growth rates and stages of sexual maturity: (1) post-hatchling size, (2) initially accelerating, relatively flat growth, (3) late accelerating growth, (4) inflection point of growth curve, (5) appearance of clitellum indicating sexual maturity, and (6) decelerating growth in fully mature animals (Reddy et al. 1992).

At each stage, oxygen consumption measurements were made for leeches starved for 48 h. The WWT of individual leeches was taken prior to the animal being placed in a computer-monitored, flow-through respirometer system (Davies et al. 1992) with the flow rate adjusted to maintain at least a 5-Torr difference (1 Torr = 133.322 Pa) between the control and the three experimental chambers. To avoid reading biased by stress of handling (Davies et al. 1992), oxygen consumption measurements were taken 1 h after introducing the animals to the system. Two activity states were defined using the impedance monitoring system: (1) random movement associated with crawling, short bursts of swimming and random probing by the leeches' anterior (termed active) and (2) no measurable activity (termed resting) (Wrona and Davies 1984). Readings from the control and each experimental chamber were taken for 20 and 40 min, respectively, and then repeated until each experimental animal provided resting and active rate measurements for at least 20 min (i.e., about 1000 replicate readings for resting and active oxygen consumption). At the end of each run, animals were freeze-dried and the dry weight (DWT) of individuals determined (Table 1). Volumetric oxygen uptake by whole animal (microlitres of oxygen per total individual DWT per hour) and weight-specific oxygen uptake (microlitres of oxygen per milligram DWT per hour) were determined following Davies et al. (1992). Aerobic scope (AS), which indicates the amount of oxygen available for aerobic respiration above maintenance, was calculated as the difference between active (Ra) and resting (Rm) weight-specific oxygen consumption (Fry 1947). Rm, Ra, and AS may be converted to energy units using an oxycaloric equivalent of 0.0202 $J\cdot\mu L\ O_2^{-1}$ (Elliott and Davison 1975).

At each life history stage, five summer and five winter *N. obscura* were tested and the double log-transformed data used in regression analysis ($n = 30$ for each group). Covariance analysis was used to test for differences between slopes. The mean Rm and Ra were calculated separately at each stage for winter and summer *N. obscura* using the appropriate regression equation. In all statistical analyses, significance is assessed at the $p < 0.05$ value.

Results

While resting and active rates of oxygen consumption (microlitres of oxygen per total individual DWT per hour) in both groups of leeches increased with body DWT (Fig. 1), the slopes for resting and active respiration were significantly different between winter and summer *N. obscura*. Conversely, weight-specific Rm and Ra (microlitres of oxygen per milligram DWT per hour) of both winter and summer *N. obscura* decreased with increase in body DWT (Fig. 2), and the slopes for Rm and Ra were significantly different between winter and summer leeches. Although stage 1 winter leeches had 64.8% higher Rm than summer leeches, the difference reduced to 10.2% by stage 6 (Table 2). Stage 1 winter leeches had 5.6% lower Ra than summer leeches, but by stage 6 the Ra of winter leeches was 20.9% higher than that of summer leeches (Table 2).

AS decreased with increase in body DWT in both winter and

TABLE 2. Calculated mean (n = 5) of oxygen consumption rates and aerobic scope (AS) (μL O_2·mg DWT $^{-1}$·h $^{-1}$) and percentage differences between winter (W) and summer (S) *N. obscura*. Rm = resting respiration rate; Ra = active respiration rate.

	Rm			Ra			AS			AS/Rm	
Stage	S	W	%	S	W	%	S	W	%	S	W
1	2.98	4.91	64.8	13.87	13.09	−5.6	10.89	8.18	−24.9	3.7	1.7
2	2.19	3.28	49.8	9.76	9.46	−3.1	7.57	6.18	−18.4	3.5	1.9
3	1.05	1.22	14.3	4.19	4.26	1.6	3.14	2.97	−5.4	3.0	2.4
4	0.81	0.90	11.1	3.15	3.37	6.0	2.34	2.47	5.3	2.9	2.7
5	0.71	0.76	7.0	2.71	2.96	9.2	2.00	2.20	9.1	2.8	2.9
6	0.59	0.65	10.2	2.15	2.60	20.9	1.56	1.95	20.0	2.6	3.0

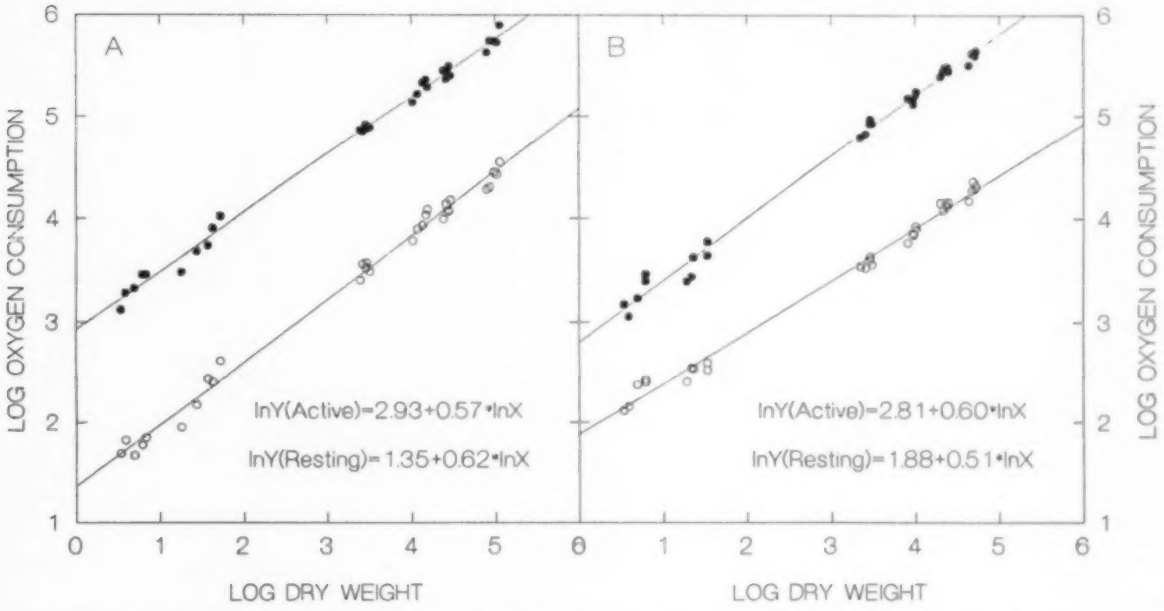

FIG. 1. Resting (open circles) and active (solid circles) rates of oxygen consumption (μL O_2· total individual DWT $^{-1}$·h $^{-1}$) in relation to DWT (mg) of (A) summer and (B) winter *N. obscura*.

summer leeches, with the slope for winter leeches significantly different from that of summer leeches (Fig. 3). While stage 1 winter *N. obscura* had 24.9% lower AS than summer leeches, by stage 6 the AS of winter leeches was 20.9% higher than that of summer leeches. Reflecting the changes in Rm and Ra, the AS/Rm ratio increased from 1.7 at stage 1 to 3.0 at stage 6 in winter *N. obscura* but decreased from 3.7 at stage 1 to 2.6 at stage 6 in summer leeches (Table 2).

Discussion

The observed differences in respiratory oxygen consumption between life history stages of *N. obscura* reflect the influence of size and/or physiological state, and the differences between winter and summer *N. obscura* reflect the influence of the winter stresses of the animals.

Levels of resting and active whole-animal oxygen consumption in summer and winter *N. obscura* are proportional to the power function of the body weight with slopes similar to those observed by Ultsch (1973) and Calow (1975). The slopes recorded by Mann (1956) for five species of British leeches were higher (0.70–1.06) than those recorded for *N. obscura*. This could be attributed to Mann's use of WWTs rather than DWTs over a restricted body weight range, the inability to determine activity-specific respiration, or interspecific differences. The decrease in weight-specific resting oxygen consumption (Rm) was asymptotic in both winter and summer *N. obscura*.

The higher Rm in winter compared with summer *N. obscura* (Table 2) probably reflects the higher costs of maintaining, mobilizing, and transporting materials for tissue repair after experiencing winter stresses. Although winter *N. obscura* maintained higher Rm than summer leeches throughout their life history, the initial decrease suggests a relatively fast metabolic recovery.

Earlier studies on the effects of stress on respiration have not measured activity-specific respiration and have looked at only one life history stage, e.g., Baird et al. (1990) inferred that Rm increased in *Daphnia magna* Straus exposed to cadmium and 3,4-dichloroaniline (DCA), and Barber et al. (1990) observed that such increases in oxygen consumption were possibly due to the costs associated with protein turnover. Maltby and Nay-

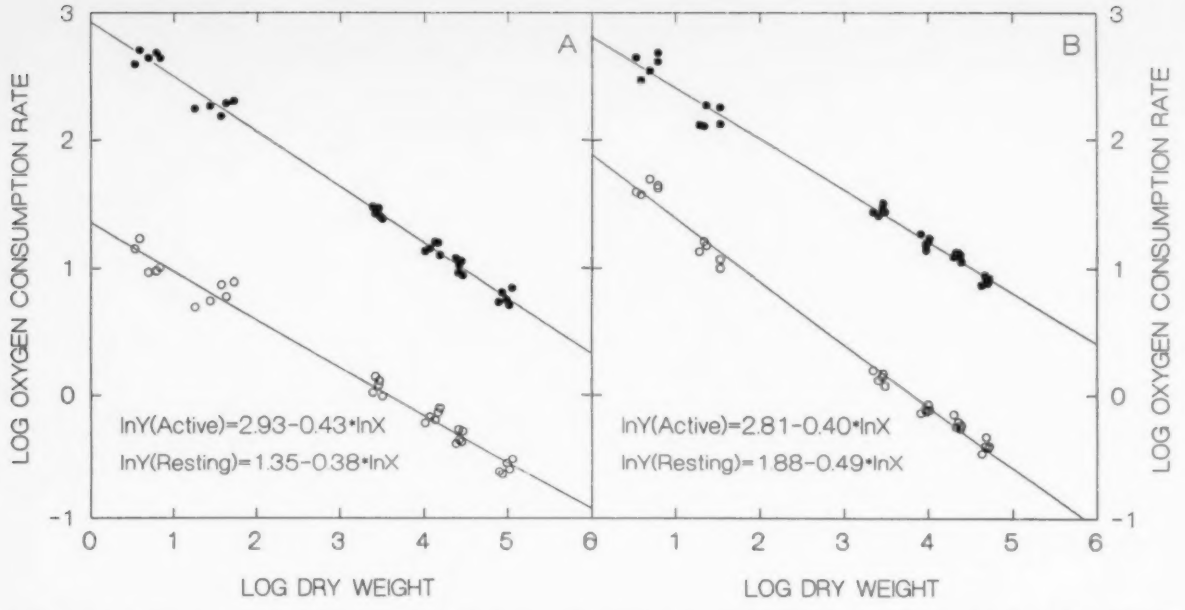

FIG. 2. Relationship between resting (open circles) and active (solid circles) weight-specific rates of oxygen consumption ($\mu L\ O_2 \cdot mg\ DWT^{-1} \cdot h^{-1}$) and DWT (mg) of (A) summer and (B) winter *N. obscura*.

lor (1990) and Maltby et al. (1990) found that zinc and DCA stresses did not result in changes in respiration by *Gammarus pulex* (L.), although exposure to ammonia did.

The significantly higher values of Ra than Rm at each life history stage of winter (2.7–4.0 times) and summer (3.6–4.7 times) *N. obscura* fall within the range (1.4–10.0) quoted by Newell (1970) for a variety of invertebrates. Overwintering resulted in lower Ra values in winter *N. obscura* during the early stages of growth, but they show a rapid respiratory recovery with overcompensation at the later stages. However, the smaller percentage differences in Ra compared with Rm in stage 1 and 2 winter leeches suggests either that Ra is less influenced by winter stresses or that there is greater ability to compensate (Table 2).

Like *N. obscura*, a decrease in AS with increase in body weight has also been observed in shrimp (*Heterosquilla tricarinata*) by Innes (1985) and some small (<10 mg wet weight) fish larvae (*Chalcalburnus chalcoides* and *Rutilus rutilus*) (Kaufmann 1990). Although AS was severely reduced following winter stress, *N. obscura* showed an ability to recover and overcompensate by stage 4.

The lower AS in stage 1, 2, and 3 winter leeches can primarily be attributed to the proportionally higher costs of Rm, while the increase and overcompensation in AS at later stages was primarily due to the proportionally higher levels of Ra. This resulted in an increase in the AS/Rm ratio with increasing body DWT in winter leeches but a decreasing AS/Rm ratio with increasing body DWT in summer leeches.

Dratnal and Davies (1990) demonstrated that winter leeches took longer to reach maturity and at a larger body size because they allocated their energy surplus primarily to lipid deposition rather than to somatic growth. This study shows that Rm of winter leeches was higher than summer leeches throughout their life history which, at least in part, explains the proportional decrease in allocation of energy to growth compared with summer leeches. The lower AS in stage 1, 2, and 3 winter leeches may indicate reduced energy potentially available for physical activities such as finding prey and suitable microhabitats and avoiding predators.

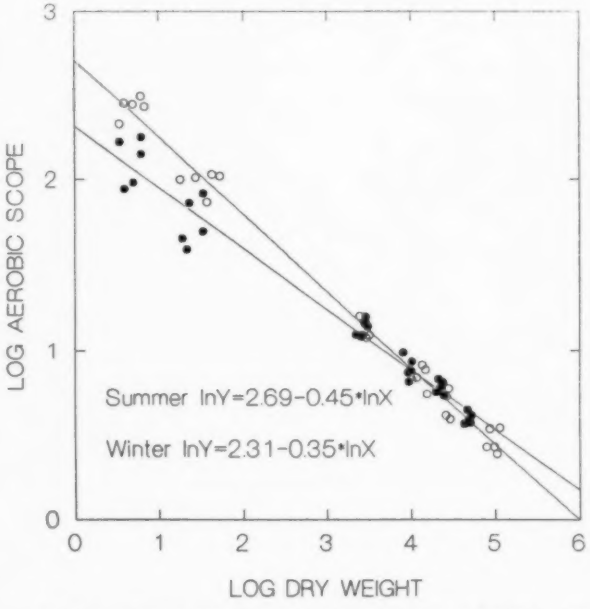

FIG. 3. Relationship between aerobic scope ($\mu L\ O_2 \cdot mg\ DWT^{-1} \cdot h^{-1}$) and DWT (mg) of summer (open circles) and winter (solid circles) *N. obscura*.

Reddy et al. (1992) demonstrated that, when transferred to optimal summer conditions, winter leeches rapidly made up the losses in total lipid and glycogen but the effects of overwintering resulted in physiological differences between winter and summer *N. obscura* throughout their life history. In this study the effects of overwintering on Rm, Ra, and AS also persisted throughout the life history of *N. obscura*, although by stage 4 the differences were small.

This is the first study demonstrating the effects of overwintering on the activity-specific oxygen consumption of a freshwater invertebrate throughout its life history. It appears that Rm is more susceptible to stress and remains higher in winter leeches throughout their subsequent life history. Compensation for Ra occurs at, or before, the inflection point of the growth curves so that at maturity, AS is higher in winter than in summer leeches.

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Effects of Biotreated Bleached Kraft Mill Effluent on Fingerling Chinook Salmon (*Oncorhynchus tshawytscha*)

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Fingerling chinook salmon (*Oncorhynchus tshawytscha*) were chronically exposed in the laboratory to nonlethal biologically treated bleached kraft mill effluent (TBKME) at concentrations and temperatures typical of the Fraser River. Laboratory exposure was for 144 d in freshwater followed by 66 d in seawater without TBKME. Exposed fish bioconcentrated chlorophenols, chloroguiacols, and extractable organochlorine substances in proportion to the aqueous concentrations of the substances. Polychlorinated dibenzo-*p*-dioxins (PCDD's) and polychlorinated dibenzofurans (PCDF's) were also bioaccumulated, but the low body burdens compared with wild fingerling chinook indicated that the laboratory environment did not account for biomagnification via the food chain. Growth, mortality, hematocrit, protein and fat content, liver somatic index, sorbitol dehydrogenase, and tolerance to hypoxia were not affected by TBKME exposure. Increased nuclear diameters of interrenal cells among TBKME-exposed fish indicated that these fish were experiencing chronic stress. Hepatic and renal granulomas of Bacterial Kidney Disease origin were observed in some TBKME-exposed fish, but there was only a tenuous link between TBKME exposure and disease resistance. Hepatic ethoxyresorufin-*O*-deethylase (EROD) activity was correlated with dioxin toxic equivalency (TEQ). From this analysis, we estimated a threshold for EROD induction between 0.3 and 1.0 ng TEQ·kg^{-1}.

Des saumons chinook (*Oncorhynchus tshawytscha*) de moins d'un an ont été exposés de manière chronique en laboratoire à des effluents d'usine de pâte kraft blanchie traitée biologiquement et non létale (EPKBT) à des valeurs de concentration et de température typiques de la situation observée dans le fleuve Fraser. Les poissons ont été exposés en laboratoire à de l'eau douce pendant 144 jours, puis à de l'eau salée sans EPKBT pendant 66 jours. On a constaté chez les poissons exposés une bioconcentration des chlorophénols, des chloroguaïacols et de composés organochlorés extractibles proportionnelle à la concentration des substances en question dans l'eau. Il y avait également bioconcentration des dibenzo-*p*-dioxines (PCDD) et de dibenzofuranes polychlorés (PCDF), mais les faibles charges corporelles observée, comparativement aux valeurs mesurées chez les jeunes chinook sauvages, ont indiqué que l'environnement de laboratoire ne tenait pas compte de la bioamplification qui survient par l'intermédiaire de la chaîne alimentaire. La croissance, la mortalité, l'hématocrite, la teneur en graisse et en protéine, l'indice somatique hépatique, l'activité de la sorbitol-déshydrogénase et la tolérance à l'hypoxie n'ont pas été affectés par l'exposition à l'EPKBT. Une augmentation du diamètre des noyaux des cellules interrénales chez les poissons exposés à l'EPKBT indique que ces poissons subissaient un stress chronique. Des granulomes hépatiques et rénaux secondaires à une néphropathie bactérienne ont été observés chez certains poissons exposés à l'EPKBT, mais le lien entre l'exposition à l'EPKBT et la résistance à la maladie était ténu. L'activité de l'éthoxy-résorufine-*O*-déséthylase (EROD) hépatique a été corrélée au degré d'équivalence toxique de la dioxine (ÉQT). À partir de cette analyse, nous avons estimé que le seuil d'induction de l'EROD se situait entre 0,3 et 1,0 ng ÉQT·kg^{-1}.

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Four bleached kraft pulp mills discharge biotreated effluents (TBKME) to the upper Fraser River at Prince George and Quesnel, British Columbia, Canada (Servizi 1989). Calculated effluent concentrations are in the range 1.5–3.0% during typical winter low-flow periods assuming bank to bank dispersion (G. Derksen, Conservation and Protection, North Vancouver, B.C., Canada, V7M 3H7, personal communication). Higher effluent concentrations would occur in dilution zones, but measurements have not been reported. The biotreated effluents have usually met acute detoxification criteria (Servizi 1989) specified by Canadian Federal Government Regulations (Environment Canada 1971, 1972) and British Columbia Provincial Government Objectives (Pollution Control Board 1977). Although unproven, it has been presumed by regulatory bodies that these criteria would assure a safe environment for aquatic life, especially the large numbers of Pacific salmon (*Oncorhynchus* spp.) common to the Fraser River. However, chemical assays revealed residual organochlorines in TBKME effluents (Voss 1984) including polychlorinated dibenzofurans (PCDF's) and dibenzo-*p*-dioxins (PCDD's) (CPPA 1989), chloroguaiacols, and chlorophenols (Voss and Yunker 1983).

Of particular interest and concern are underyearling chinook salmon that overwinter in the upper Fraser River mainstream. Underyearling chinook captured in the upper Fraser River contained organochlorines of bleached kraft mill origin (Rogers et al. 1988), and hepatic mixed function oxidase (MFO) levels were elevated (Rogers et al. 1989). Although depressed returns of chinook salmon to the Fraser River are believed to be related primarily to increased harvest, there is concern that contaminant residues and elevated MFO levels may compromise survival of chinook salmon. Accordingly, a laboratory study was conducted in the period January–August 1989 using fingerling chinook to detect possible impacts of chronic TBKME exposure in freshwater and postexposure residence in seawater. We applied a suite of tests, as determined from review of the scientific literature, which included survival, growth, proximate analysis, hepatic ethoxyresorufin-*O*-deethylase (EROD) activity, serum sorbital dehydrogenase (SDH), hematocrit, liver somatic index (LSI), hypoxia tolerance, and histopathology. In addition, specific organochlorine body burdens were measured, since these were detected in wild chinook fingerlings and are known toxic components of nonlethal TBKME. Acute bioassays of TBKME were made to verify nonlethality.

Methods

Bleached Kraft Mill Effluent

Biotreated BKME was obtained from an upper Fraser River pulp mill which discharges effluent from an aerated lagoon (approximately 8 d of retention). Effluent was collected at the lagoon outlet and shipped the same day by overnight express 4 d per week during the first 60 d in 22.5-L plastic containers filled to the top and capped. From day 60 to 144, TBKME was collected and shipped once per week. Upon arrival at the laboratory, the TBKME was used immediately or stored at 2°C in the dark for 1–6 d before use. Four-litre aliquots of TBKME from each shipment were preserved at pH 2 (H_2SO_4) in amber glass bottles filled to the top and sealed with aluminum caps (methanol rinsed) for future analysis.

Acute Bioassay

Nine samples were reserved for static acute bioassays, which were conducted using 30 L of undiluted TBKME in glass aquaria. Aeration was supplied via oil-free compressed air to maintain dissolved oxygen near saturation. Temperatures were in the range 6–9°C. Ten fingerling chinook salmon (mean 1.7 g) were exposed for 96 h. A parallel control using Cultus Lake water was set up for each test.

Chronic Bioassay

Following acclimation to test apparatus and temperatures, fingerling chinook were exposed to TBKME in the laboratory at concentrations and temperatures representative of the upper Fraser River in winter and spring. When a control group survived a seawater tolerance test, indicating their smoltification had occurred, the fish were transferred to seawater free of TBKME. Details are described below.

Chinook Salmon

Eyed chinook salmon eggs from the upper Fraser River (Bowron River stock) were transferred from Quesnel River Hatchery to Cultus Lake Laboratory. Incubation continued in Cultus Lake water (Servizi and Martens 1991) and fry were fed Biodiet starter. Fingerlings were fed Oregon Moist Pellets (OMP) at the daily rate of 1.5% of body wet weight prior to testing. Fish were transferred from laboratory stocks to five 1300-L cylindrical fibreglass tanks filled with Cultus Lake water. The five groups (A through E) of 202 fingerling chinook each were acclimated for 10 d to daily (except weekend) drain and refill (95% replacement) procedures using water only. During the first 5 d of acclimation, temperature was lowered from 3 to 0.9°C. Thirty-two fish in each of groups A through D were previously implanted with passive integrated transponder (PIT) tags so that individual weights could be tracked throughout the study. Group E was only sampled near the time of smoltification to test for seawater readiness using osmoregulatory capacity as a criterion. Osmoregulatory capacity was measured according to Blackburn and Clarke (1987) except that serum chloride levels were measured. Seawater adaption was confirmed when serum chloride levels were 143.8 meq·L^{-1} or less (J. Blackburn, Pacific Biological Station, Nanaimo, B.C., Canada, V9R 5K6, personal communication).

Each of the fibreglass tanks was fitted with four 3160 cm^2 × 1 mm thick aluminum panels for heat transfer to an outer continuous-flow water cooling jacket. Each tank was equipped with eight air diffusers that supplied oil-free compressed air to maintain oxygen saturation and provide circulation. Tanks were covered with tanslucent lids.

TBKME Exposure

Following acclimation and baseline sampling, TBKME was added to groups A and B at 4 and 1.5% (v/v), respectively. Drain and refill procedures required 30 min per tank. Exposure continued for 60 d at these concentrations at a mean temperature of 0.9°C (SD = 0.39) and a pH of 7.80 (0.13). Control groups (C, D, E) followed a similar routine but without TBKME (Table 1). Food was withheld for the first 40 d, since metabolic demands were minimal and field studies indicated that overwintering fingerling chinook were probably not foraging. Thereafter, fish were fed OMP at approximately 0.1% of body weight once per week to maintain digestion. At 60 d, all except group E were sampled for a suite of tests described later.

TABLE 1. Schedule for exposure of fingerling chinook salmon to TBKME (%, v/v).

Group	Exposure interval (d)		
	1–60 (0.9°C)	61–144 (1–12°C)	145–210 (13.2°C)
A	4	0.8	0[a]
B	1.5	0.3	0
C	0	0	0
D	0	0	0
E	0	0	0

[a]Pit-tagged fish from groups A, B, C, and D transferred to seawater at the start of this interval. All non-pit-tagged fish were used for sampling prior to seawater transfer.

After 60 d, the remaining fish (approximately 120 per group except 202 in E) were transferred to continuous-flow fibreglass troughs where groups A and B were exposed to 0.8 and 0.3% (v/v) TBKME, respectively, for 84 d from day 61 to 144 (Table 1). The lower concentrations were consistent with mean TBKME concentrations in the Fraser River during spring. Control groups (C, D, E) were transferred to similar troughs without TBKME. Temperatures were gradually increased from 1 to 12°C, as would occur in the Fraser River, during the 84-d exposure. Fish were fed OMP twice daily at a daily rate of 1% of body weight.

Troughs were 300 × 36 cm wide and contained 186 L. TBKME was pumped from a reservoir to a gravity-flow dilution apparatus that used calibrated metering tubes to deliver the appropriate amounts of effluent and dilution water (Cultus Lake water) to each trough. A flow rate of 1 $L \cdot min^{-1}$ was used in experimental and control troughs. This flow provided 90% volume replacement in 7 h and a loading of about 2.2 $L \cdot g^{-1} \cdot d^{-1}$ as recommended by Sprague (1969). Oil-free compressed air was added to each trough to ensure adequate dissolved oxygen and as a precaution in the event of pump failure.

Osmoregulatory testing of group E indicated that fingerlings were ready to accept seawater at about day 144. Accordingly, the non-PIT-tagged fish in groups A, B, C, and D were sampled for analyses. The PIT-tagged fish from these groups were weighed and measured and transferred to a 950-L seawater tank at Vancouver Public Aquarium (VPA). This transfer represented migration from the Fraser River to seawater. Accordingly, TBKME was no longer added. The seawater phase continued from day 145 to 210 (Table 1). The seawater tank was supplied with sand-filtered water at 24 $L \cdot min^{-1}$ to yield 99% replacement in 3.5 h. Mean temperature was 13.2°C (range 12–15°C), chlorinity was 15 $g \cdot kg^{-1}$, pH was 7.80, and dissolved oxygen was 90% saturated. The fish were fed OMP once daily at a rate of 1% of body weight.

Prior to TBKME exposure and at 60 and 144 d, fish were sampled for weight, length, proximate analysis, extractable organic chlorine (EOCL), specific organochlorine contaminants (including dioxins and furans), hepatic EROD, serum SDH, hematocrit (% PCV), LSI, hypoxia tolerance, and histopathology. At 210 d, fish were sampled for all of the above except for SDH, LSI, proximate analysis, and hypoxia tolerance.

Wet weight was measured to the nearest 0.01 g on a digital top-loading pan balance (Mettler PR700). Length was measured to 0.1 cm from the snout to the fork of the tail. Specific growth rates were calculated according to Busacker et al. (1990). LSI was calculated as the ratio of liver wet weight immediately after excision and fish total wet weight. Blood for PCV was collected in 75 × 1.1 mm (inside diameter) heparinized microhematocrit tubes after caudal peduncle severance. Tubes were iced, immediately centrifuged at 13 000 × *g* force for 3 min (International model MB), and measured on a reader (International model CR). After measurement, that portion of the tube containing serum was broken off, sealed (Seal-Ease, Clay-Adams), and sent via air express (same-day service) on dry ice to the Freshwater Institute (Winnipeg, Man.) where SDH was measured using a dehydrogenase test kit (Sigma-50UV). Reagent volumes were adjusted to suit the small volumes of serum (50 μL). Tolerance to hypoxia was measured using one fish per water-sealed bottle (0.5 L). Ten fish were tested from each group. Residual oxygen at cessation of opercular movement was measured with an oxygen meter (YSI model 58) fitted with a self-stirring probe.

Proximate analyses (moisture, fat, protein, and ash) were made according to Anonymous (1984). Ten fish from each group were analysed at each sampling time.

Histopathology samples consisting of 10 fish from each group were taken at 0, 60, 144, and 210 d. Each fish was stunned, the caudal peduncle was severed to facilitate blood collection, and gill arches and samples of liver, kidney, and head kidney were removed and preserved in Bouin's fluid. Following embedding in paraffin, 5-μm sections of liver, kidney, head kidney, and gill tissue were stained with hematoxylin and eosin. Interrenal nuclear diameters (IRND) were measured in head kidney sections using a light microscope. For each fish, five IRND measurements were made of each of four different areas giving a total of 200 measurements per group at each sampling (McBride and Overbeeke 1969). Classification and interpretation of presumptive tumors were done by Dr. Michael Kent, Fish Disease Laboratory, Pacific Biological Station, Nanaimo, B.C., Canada.

Chlorophenols, chloroguaiacols, and chlorocatechols in TBKME and tissues were measured by electron capture gas chromatography (Rogers et al. 1988). PCDD and PCDF residues in tissues were measured by GC-MS using $^{14}C_{12}$-labelled internal standards according to the procedures described in Norstrom et al. (1990). The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalency values (TEQ) for mixtures of dioxins and furans, expressed as nanograms per kilogram, were calculated according to toxicity equivalency factors (TEF) that are relative to the acute toxicity of 2,3,7,8-TCDD (Walker and Peterson 1991). PCDD's and PCDF's in TBKME were measured according to methods of Lamparski and Nestrick (1980) and Smith et al. (1984).

Hepatic EROD activity was determined at 25°C by a fluorometric procedure based on the methods of Pohl and Fouts (1980). Livers were immediately excised from stunned fish, quickly wrapped in methanol-washed aluminum foil, placed in a plastic bag, and promptly frozen on dry ice until analysed. There was insufficient liver to prepare microsomal suspensions; therefore, postmitochondrial supernatants were prepared from each liver. EROD activity was measured as nanomoles of resorufin produced per milligram of protein per minute. Protein content was measured using the Lowry method as modified by Markwell et al. (1981). Relative EROD activity was calculated by dividing each EROD value by the mean EROD for the control groups at the corresponding sampling time.

Statistical Analysis

Analysis of variance was computed by the GLM procedure

TABLE 2. Acute toxicity, chemical characteristics, and discharge to TBKME.

	n	Mean	Max.	Min	SD
Acute toxicity (% mortality)	9	0	0	0	
pH	40	7.53	8.67	7.21	±0.26
Conductivity at 25°C ($mS \cdot m^{-1}$)	40	336	387	290	23
Resin acids ($mg \cdot L^{-1}$)	7	0.36[a]	1.24	Trace	—
2,4,6-Chlorophenol ($\mu g \cdot L^{-1}$)	46	6.8	10.5	2.5	1.5
Tetrachlorophenol ($\mu g \cdot L^{-1}$)	46	2.5	3.9	0.3	0.7
Pentachlorophenol ($\mu g \cdot L^{-1}$)	46	1.1	1.8	0.6	0.3
3,4,6-Trichloroguaiacol ($\mu g \cdot L^{-1}$)	46	8.1	12.9	2.3	2.2
3,4,5-Trichloroguaiacol ($\mu g \cdot L^{-1}$)	46	41.0	81.3	18.7	14.3
4,5,6-Trichloroguaiacol ($\mu g \cdot L^{-1}$)	46	8.5	14.9	3.7	2.4
Tetrachloroguaiacol ($\mu g \cdot L^{-1}$)	46	44.3	92.7	8.7	15.7
Tetrachlorocatechol ($\mu g \cdot L^{-1}$)	46	24.8	61.2	1.9	13.4
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin ($ng \cdot L^{-1}$)	6[b]	<0.03	0.043	<0.03	0.004
2,3,7,8-Tetrachlorodibenzofuran ($ng \cdot L^{-1}$)	6[b]	0.417	0.66	0.20	0.18
Adsorbable organochlorine ($kg \cdot ADt^{-1}$)	6	3.1	3.80	2.30	0.49
Adsorbable organochlorine ($kg \cdot d^{-1}$)	6	4480	5540	3291	743
TBKME discharge ($m^3 \cdot d^{-1}$ (x 10^{-3}))	140	147	164	134	11

[a]Assuming trace as zero.
[b]Six pooled samples made of 4–16 TBKME aliquots per pool.

and means were compared using Tukey's Studentized range test (SAS Institute Inc. 1985).

Results

TBKME Characteristics

Although the effluent had been biologically treated in an aerobic system, it contained residual resin acids, plus an assortment of chloroorganics, including TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Table 2). The sum of identified chloroorganics equals 137 $\mu g \cdot L^{-1}$, which, when multiplied by effluent flow, yields a mean daily discharge of 20.1 $kg \cdot d^{-1}$. The daily discharge of adsorbable organochlorine (AOX) was 4216 $kg \cdot d^{-1}$. Thus, only about 0.48% of the daily AOX discharge was in the form of identified chloroorganics. No mortalities occurred among any fish during 96-h exposures to 100% (v/v) TBKME or among Cultus Lake water controls (Table 2).

Bioconcentration

Although tetrachlorocatechol was present in TBKME, none was detected in tissue. On the other hand, EOCL, total chlorophenols, and total chloroguaiacols were bioconcentrated in proportion to TBKME exposure levels (Fig. 1). No polycyclicaromatic hydrocarbons (PAH's) were detected in any of the fish.

Chlorinated dioxins and furans also composed a portion of the organochlorine body burden (Table 3). The 2,3,7,8 congener was the only dioxin detected and the levels in TBKME-exposed groups were no higher than the minimum detectable concentration (MDC). This congener was not detected in the preexposure sample or the control groups (C and D) at 144 and 210 d, but at 60 d, there was 2 $ng \cdot kg^{-1}$ in group C and a trace in group D. The former value appears high compared with the other control group measurements and may reflect analytical variability at the MDC. The only furans detected in fish tissues included the 2,3,7,8 congener and an unresolved mixture of 2,3,4,7,8 and 1,3,4,8,9 congeners. The former congener

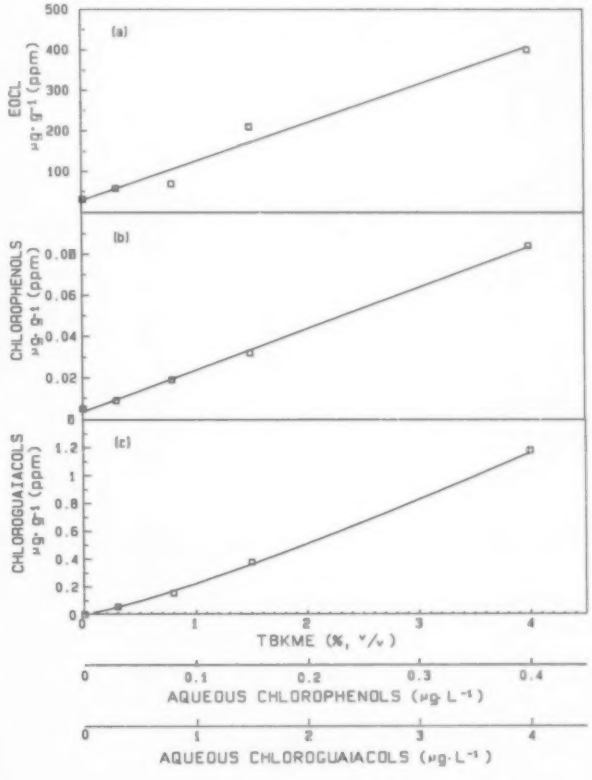

FIG. 1. Body burdens of (a) EOCL, (b) total chlorophenols, and (c) total chloroguaiacols in fingerling chinook during exposure to TBKME. Aqueous chlorophenol and chloroguaiacol exposure concentrations are the product of percent TBKME exposure and total mean concentrations of either chlorophenols or chloroguaiacols summed from Table 2.

TABLE 3. Polychlorinated dibenzo-*p*-dioxin, dibenzofuran body burdens, and 2,3,7,8-TCDD toxic equivalency (TEQ) in control and TBKME-exposed groups of fingerling chinook salmon ($ng \cdot kg^{-1}$ = ppt) (single analyses of pooled samples). n.d. = not detected (signal/noise < 2).

Group	No. in pool	Elapsed time (d)	2,3,7,8-TCDD ($ng \cdot kg^{-1}$)[a]	2,3,7,8-TCDF ($ng \cdot kg^{-1}$)	2,3,4,7,8-PCDF, 1,3,4,8,9 PCDF ($ng \cdot kg^{-1}$)	TEQ ($ng \cdot kg^{-1}$)
Preexposure	10	0	n.d.	6	n.d.	0.17
A	10	60	2	11	n.d.	2.31
B	10	60	2	11	n.d.	2.31
C	10	60	2	8	n.d.	2.22
D	10	60	tr	6	n.d.	0.17
A	10	144	2	2	n.d.	2.06
B	10	144	n.d.	6	4	1.60
C, D[b]	10	144	n.d.[b]	3[b]	n.d.[b]	0.08
A	6	210	n.d.	3	n.d.	0.08
B	—[c]	210	—[c]	—[c]	—[c]	
C, D[b]	10	210	n.d.[b]	9[b]	n.d.[b]	0.25
MDC[d]			2	2	3	
TEF[e]			1.0	0.028	0.359[f]	

[a]The following eight PCDD congeners were not detected: penta (1), hexa (5), hepta (1), and octa (1). Analyses were made for nine PCDD congeners and three PCDF congeners.
[b]Fish from groups C and D were pooled for analysis.
[c]No sample.
[d]Minimum detectable concentration at signal/noise = 3. Probable detection at amounts less than the MDC listed as trace (tr).
[e]Toxicity equivalency factor (Walker and Peterson (1991).
[f]TEF value for 2,3,4,7,8-PCDF only.

TABLE 4. Percentage of identified organochlorine substances in the EOCL body burdens of fingerling chinook salmon.

		Body burdens		
Group	Elapsed time (d)	Total identified organochlorine contaminants ($\mu g \cdot g^{-1}$ (ppm)	EOCL ($\mu g \cdot g^{-1}$ (ppm))	EOCL identified (%)
Preexposure		0.005	31	0.015
A	60	1.264	400	0.320
B	60	0.410	210	0.200
C	60	0.004	48	0.009
D	60	0.004	82	0.005
A	144	0.173	69	0.251
B	144	0.065	58	0.110
C	144	0.003	26[a]	0.0001
D	144	0.001	—[a]	0.004
B	210	—[b]	24	
D	210	—[b]	25	

[a]Fish from groups C and D were combined for analysis.
[b]No measurement.

generally exceeded the detection limit and was at its highest level, 11 $ng \cdot kg^{-1}$, in groups A and B after 60 d of exposure to TBKME. Statistical comparisons of body burdens among the groups were not possible because single pooled samples were used for analyses owing to the small size of the fish.

The TEQ values for TBKME-exposed groups A and B at 60 d were 2.31 $ng \cdot kg^{-1}$ but decreased at 144 d when TBKME exposure decreased and declined to background levels at 210 d after TBKME exposure ended (Table 3). The TEQ for group B at 144 d was caused largely by the PCDF content. The TEQ values at 60 d for control groups C and D were 2.22 and 0.17 $ng \cdot kg^{-1}$, respectively, but the higher TEQ for C was caused primarily by a 2 $ng \cdot kg^{-1}$ body burden of 2,3,7,8-TCDD and may be the consequence of analytical variability at the MDC.

The organochlorine substances identified as portions of the body burdens of fingerling chinook (Fig. 1b and 1c; Table 3) were summed and divided by the total EOCL derived from Fig. 1a to obtain the proportions of identified EOCL (Table 4). For TBKME-exposed groups, only 0.11–0.32% of EOCL were identified. For preexposed and control groups (C and D), these percentages ranged from 0.0001 to 0.005. For both experimental and control groups, virtually all of the organochlorine body burden was not identified.

Physiological and Biochemical Responses

Moisture content increased slightly from 0 to 60 d for all groups (Fig. 2), while fat and protein contents decreased and ash content was unchanged; this is not surprising because moisture content rises when no food is offered. There were no significant differences in proximate analyses among the four groups at 60 or 144 d, except that ash content of group A was greater than that of group D (ANOVA, $p = 0.05$).

Hematocrit was consistent and unaffected by TBKME exposure (Fig. 3a). There were no significant differences in hematocrit among the groups at 60 and 210 d. However, after 144 d of exposure, control group C was different from the other three groups ($p = 0.05$). Liver somatic index was not affected by TBKME exposure (Fig. 3b). The residual dissolved oxygen concentrations at which fish perished during measurements of hypoxia tolerance were not affected by TBKME exposure except for group A which differed from control group C after 60 d of exposure (Fig. 3c). Control group D did not support this finding. Control groups C and D were significantly different from each other but not from group A and B after 144 d of exposure. Residual dissolved oxygen levels were uniformly higher at 144 d than at 60 d. These differences were believed due to the higher test temperatures (11.9°C) at 144 d causing a greater metabolic demand for oxygen than at 60 d (1°C). SDH at 60 d was less for all groups than the preexposure level, but after 144 d, SDH levels returned to preexposure levels (Fig. 3d) At 60 d, SDH was lower in groups A and B than in the two control groups, and group A was different from C (ANOVA, $p < 0.05$). However, this difference was not confirmed by control group D.

EROD Activity

After 60 and 144 d of exposure to TBKME, hepatic EROD activities in groups A and B were significantly elevated above control levels (Fig. 4). At 210 d, after 66 d in seawater without TBKME, EROD activities in groups A and B were similar to control levels. EROD activities of the two control groups increased from preexposure to 60 and 210 d, but this was expected because the physiological changes accompanying growth and smoltification could influence EROD activity.

Histopathology

There was no evidence of pathology in any of the gill tissue. However, 7 of 10 livers and 1 of 5 kidneys from fish exposed to TBKME at 144 d contained large microscopic circular lesions (300–400 μm). The lesions were diagnosed as granulomas and not tumors. Furthermore, appearance indicated that the lesions were caused by Bacterial Kidney Disease (BKD), but the low numbers of associated bacteria suggested that the lesions were healing. The analyst estimated that the infection that caused the lesions had occurred several weeks earlier. Because exposure to TBKME commenced 20 wk prior to sampling at 144 d, the BKD infection may have occurred during the study. There were, however, no outward signs of disease during the first 144 d.

Interrenal Nuclear Diameter

IRND's were larger than control values (ANOVA, $p = 0.05$) for group A at 60 and 144 d (Table 5). The increases were 5.1 and 1.6%, respectively, and coincided with TBKME exposure concentrations of 4 and 0.8% (Table 1). Group B IRND were

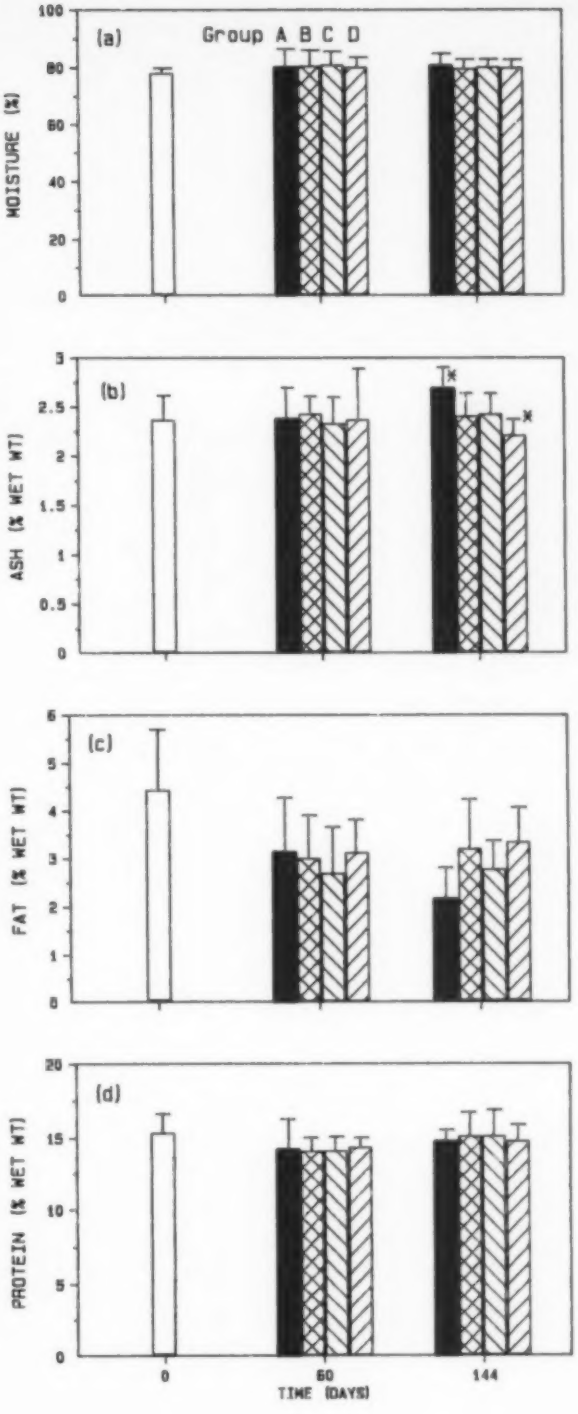

FIG. 2. Proximate analyses of TBKME-exposed (groups A and B) and control (groups C and D) fingerling chinook salmon. Groups marked with an asterisk are significantly different at each sample time ($p = 0.05$).

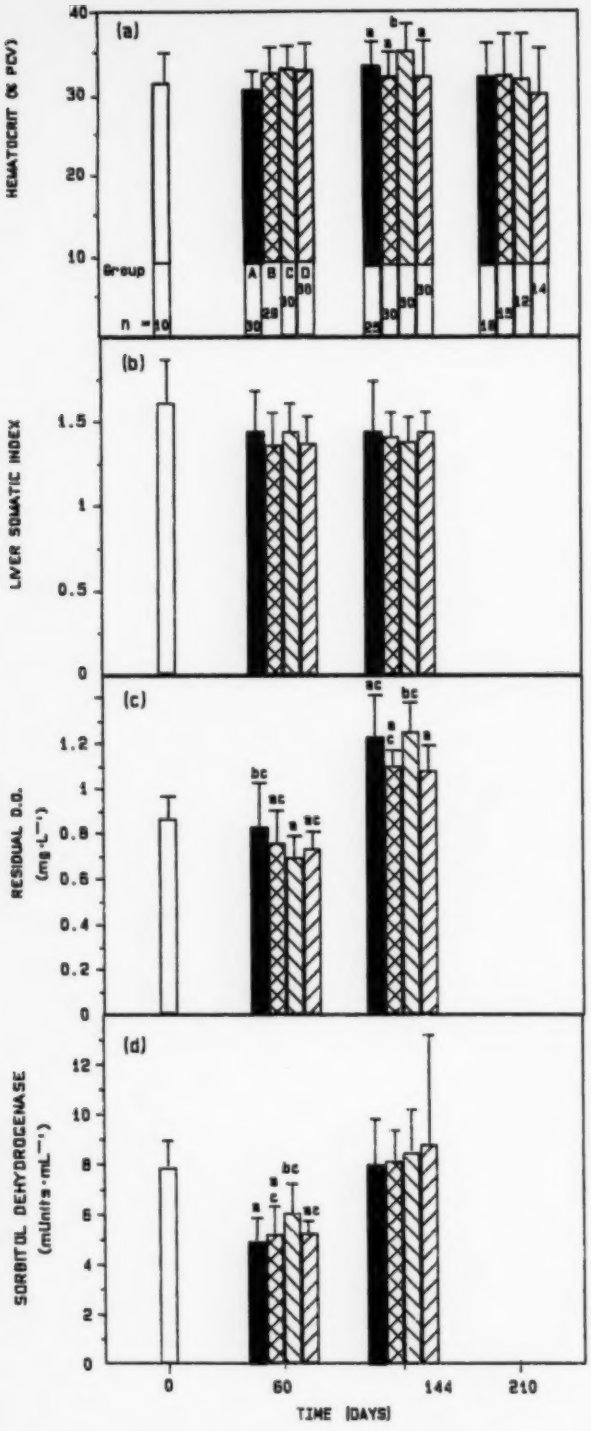

FIG. 3. Mean (a) hematocrits, (b) LSI, (c) residual dissolved oxygen at hypoxic death, and (d) SDH among TBKME-exposed (groups A and B) and control (groups C and D) fingerling chinook salmon. Groups with the same letter are not significantly different at each sample time ($p = 0.05$).

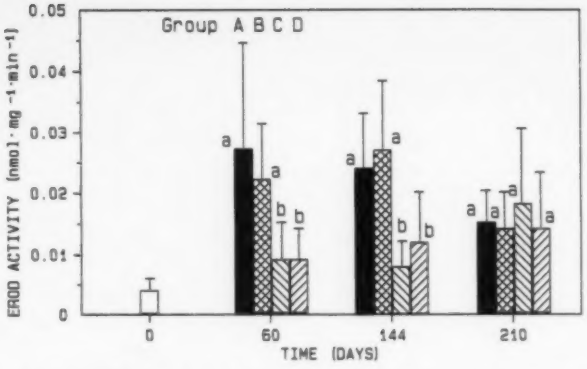

FIG. 4. Mean hepatic EROD activity for TBKME-exposed (groups A and B) and control (groups C and D) fingerling chinook salmon. Groups with the same letter are not significantly different at each sample time ($p = 0.05$).

TABLE 5. Mean (SE) interrenal nuclear diameters (μm) of fingerling chinook salmon sampled from control and TBKME-exposed groups. Sample size was 10 throughout. For each elapsed time, values with a common letter are not significantly different.

	Elapsed time (d) from start of TBKME exposure			
Group	0	60	144	210
Preexposure	6.009 (0.037)			
A		6.534 a (0.036)	5.964 b (0.019)	6.020 a (0.016)
B		6.316 b (0.036)	5.869 b (0.020)	5.990 a (0.016)
C		6.194 c (0.036)	5.870 b (0.020)	6.010[a] a (0.016)
D		6.238 bc (0.036)	5.871 b (0.019)	

[a]Composed of five fish from each of groups C and D.

1.9% larger than control C when sampled at the end of 60 d of exposure to 1.5% (v/v) TBKME, but the difference between group B and control D was not significant. Furthermore, there were no significant differences between group B and control groups at 144 d following exposure to 0.3% (v/v) TBKME. No differences occurred among IRND values after the fish had been in seawater in the absence of TBKME for 65 d. Among control groups, IRND values were greatest after 60 d at 0.9°C.

Growth and Mortality

There were no significant differences among mean weights and lengths of the PIT-tagged fish in groups A, B, and C at day 0 (Fig. 5). The day 0 weight and length data for group D were lost. Although temperatures were low and fish had not been fed for 51 d since acclimation started, fish responded quickly to food when once-weekly rations commenced at 40 d of exposure. Vigorous feeding continued when daily rations were provided starting at 61 d of exposure. Significant differences ($p = 0.05$) in mean weights or lengths occurred at 69 and 105 d. At 69 d, control group C was slightly heavier than the other groups and the length was greater than that of control group D. At 105 d, the mean weight of control group D was

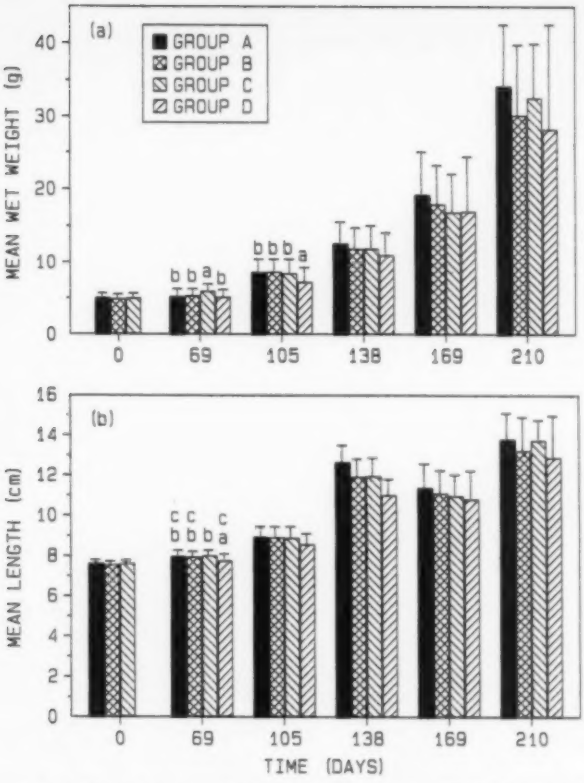

FIG. 5. Mean (a) wet weights and (b) lengths for TBKME-exposed (groups A and B) and control (groups C and D) fingerling chinook salmon. Groups with the same letter are not significantly different at each sample time ($p = 0.05$).

less than that of any of the other groups. Growth was substantial after daily food rations were resumed and temperatures were increased (60 d). Six days before the TBKME exposure period ended at 138 d, weight and length of experimental and control groups had increased approximately 2.4 and 1.6 times, respectively, compared with day 0.

During the first experimental interval (0–69 d, Table 6), specific growth rate based on weight (G_W) was greater for control group C than for the TBKME exposure groups, but there was no difference for length-based specific growth rate (G_L). In the second interval, G_W was significantly greater for TBKME exposure groups (A and B) than for controls (C and D), while there were no differences in values of G_L. In the third interval, G_W's for groups A and D were larger than for B and C, while G_L was greater for group A than for B and C, but there was no difference between A and D. Specific growth rates during the fourth and fifth intervals were similar. There were no indications from the foregoing growth comparisons to indicate a consistent correlation between TBKME exposure and specific growth rates.

Mortality

Mortalities were low during the first 144 d when fish were exposed to TBKME. A total of 11 and 10 fish in groups A and B, respectively, died during this period compared with 5 and 12 in the two control groups (C and D). Mortalities were greater

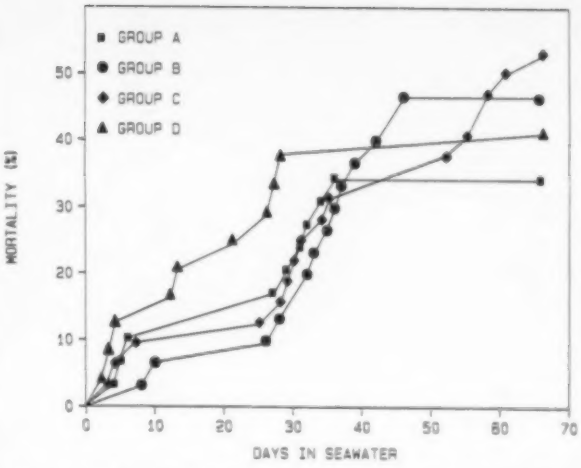

FIG. 6. Mortalities of fingerling chinook salmon after transfer to seawater.

in seawater than in freshwater. The number that died in seawater was 10 and 17 for groups A and C, respectively, and corresponded to 34.4 and 53.1%. Overall, 21 and 24 fish died in groups A and B, respectively, while 22 died in each of the control groups. The causes of mortalities in seawater were not diagnosed, but dark color and bulging eyes on many of the fish suggested BKD. Mortalities were low shortly after transfer to seawater, but started to increase among group D (Fig. 6). Subsequently, starting at about 25 d, mortalities began to increase sharply among the other three groups. Since these PIT-tagged fish were in the same tank, it seems plausible that disease may have started in group D and then spread to the other three groups. There was no indication from the foregoing data that mortalities and TBKME exposure were correlated.

Discussion

The absence of mortalities during acute bioassays of TBKME is compatible with the chemical analyses which showed that toxic chemicals were below known lethal levels. Specifically, total resin acids, the primary toxic constituents of BKME, averaged only 0.36 $mg \cdot L^{-1}$ after biological treatment, well below the 2 $mg \cdot L^{-1}$ lethal level reported for salmonids (Leach and Thakore 1973; Rogers 1973). Total chlorophenols (10.4 $\mu g \cdot L^{-1}$) averaged about 10% in the lethal level measured for swim-up pink salmon fry (*Oncorhynchus gorbuscha*) (Servizi et al. 1988). The mean tetrachlorocatechol concentration was 3% of the 96-h LC50 for sockeye (*Oncorhynchus nerka*) fry and smolts (Servizi et al. 1968). Chloroguaiacols averaged between 8 and 14% of 96-h LC50 values for rainbow trout (*Oncorhynchus mykiss*) (Leach and Thakore 1973). The mean TBKME concentrations 2,3,7,8-TCDD and 2,3,7,8-TCDF were less than 80% and approximately 11%, respectively, of the long-term exposure lethal levels following chronic exposure for 56 and 28 d (Mehrle et al. 1988). In addition to being nonlethal the TBKME used can be considered typical, since resin acids, total chlorophenols, total chloroguaiacols, and total catechols fall between values reported for two other TBKME's (Anonymous 1990; Hall et al. 1991). Finally, AOX averaged 3.1 $kg \cdot ADt^{-1}$, which was typical of modern bleached kraft pulp mills at the time (L. Adamache, Conservation and

TABLE 6. Mean specific growth rates for weight (G_w) and length (G_L) and SD calculated from individual specific growth rates for PIT-tagged chinook salmon. Values with the same letter in each time interval are not significantly different ($p = 0.05$).

Group	Time interval (d) 0–69	69–105	105–138	138–169	169–210
			G_w ($g \cdot d^{-1}$)		
A	0.049 b	1.318 a	1.206 a	1.326 a	1.221 a
SD	0.197	0.289	0.206	0.438	0.369
n	31	28	28	23	16
B	0.128 b	1.310 a	0.923 b	1.221 a	1.184 a
SD	0.181	0.338	0.254	0.460	0.275
n	32	30	29	25	15
C	0.259 a	0.905 b	1.014 b	0.983 a	1.222 a
SD	0.175	0.404	0.280	0.446	0.292
n	32	32	32	26	11
D		0.876 a	1.249a	1.085 a	1.021 a
SD		0.346	0.241	0.486	0.449
n		24	24	15	13
			G_L ($cm \cdot d^{-1}$)		
A	0.066 a	0.309 a	0.405 a	0.369 a	0.394 a
SD	0.052	0.112	0.085	0.124	0.129
n	31	28	28	23	16
B	0.074 a	0.324 a	0.336 b	0.311 a	0.407 a
SD	0.050	0.103	0.093	0.128	0.068
n	32	30	29	25	15
C	0.073 a	0.288 a	0.321 b	0.290 a	0.419 a
SD	0.036	0.078	0.069	0.104	0.042
n	32	32	32	26	11
D		0.270 a	0.346 ab	0.302 a	0.383 a
SD		0.127	0.088	0.143	0.171
N		24	24	15	13

Protection, North Vancouver, B.C., Canada V7M 3H7, personal communication).

Total chlorophenol and chloroguaiacol concentrations in a well-mixed section of the Fraser River downstream of pulp mills at Prince George averaged 0.182 and 0.796 $\mu g \cdot L^{-1}$, respectively, in March 1987 (Rogers et al. 1988). Chlorophenol and chloroguaiacol body burdens in fingerling chinook salmon averaged 0.01 and 0.12 $\mu g \cdot g^{-1}$, respectively, for samples collected in November 1986 at the same place as the water samples (Rogers et al. 1988). These values are compatible with the aqueous concentrations and body burdens reported for this laboratory study (Fig. 1b and 1c) and indicate that laboratory and field exposures were comparable. Further evidence that chlorophenol body burdens can be approximated from aqueous concentration data can be seen by comparing chlorophenol body burdens in Fig. 1b, which span a fivefold range, with a larger data set for salmonids spanning about 2.5 orders of magnitude and described by the equation (Servizi et al. 1988)

$$(1) \qquad \ln(y) = 6.03 + 1.22 \ln(x)$$

where y is chlorophenol body burden (nanograms per gram) and x is concentration of chlorophenols in water (micrograms per litre). Equation 1 predicts a body burden of 19 $ng \cdot g^{-1}$ at an aqueous chlorophenol concentration of 0.08 $\mu g \cdot L^{-1}$, while the measured value from Fig. 1b is also 19 $ng \cdot g^{-1}$ (0.019 $\mu g \cdot g^{-1}$) at this aqueous concentration. At an aqueous concentration of 0.16 $\mu g \cdot L^{-1}$, the predicted and measured values are 44 and 32 $ng \cdot g^{-1}$, respectively. The new data set appears compatible with previously published values.

The 2,3,7,8-TCDD and 2,3,7,8-TCDF body burdens of fingerling chinook salmon collected from the Fraser River downstream of pulp mills were 68 and 370 $ng \cdot kg^{-1}$, respectively in April 1988 (Rogers et al. 1989). These dioxin and furan body burdens are more than 30 times those measured in fish exposed to TBKME in the laboratory (Table 3) in spite of laboratory TBKME concentrations being in the same range as those in the Fraser River. We believe that comparison of the field and laboratory data shows that aqueous uptake is not a major factor in bioaccumulation of dioxins and furans. Uptake via the food chain (biomagnification) is the alternative route, as suggested by Rogers et al. (1989), and would appear to be the dominant bioaccumulation mechanism.

Chronic in vitro exposure to TBKME at environmentally relevant temperatures, concentrations, and exposure times had no significant effect on fingerling chinook mortality, growth, SDH, haematocrit, LSI, tolerance to hypoxia, and proximate analysis. On the other hand, hepatic granulomas suggested a possible disease link to chronic TBKME exposure. Similarly,

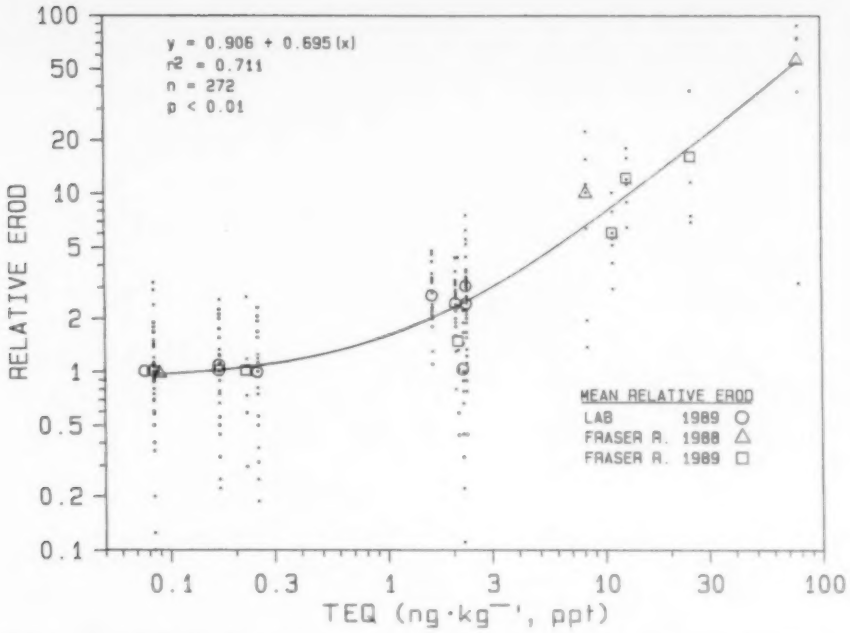

FIG. 7. Hepatic EROD activities and TEQ body burdens for fingerling chinook salmon exposed to TBKME in the laboratory and collected from the Fraser River. Relative EROD values were derived from individual measurements of 272 fish. TEQ values are based on single composite samples.

in a 60-d laboratory study using rainbow trout, mortality and growth were unaffected by exposure to 2 and 4% BKME, but there was an apparent reduction in resistance to bacterial fin rot disease (Couillard et al. 1988); no histological lesions were observed in the liver or kidney. Since hepatic granulomas were diagnosed as evidence of BKD among TBKME-exposed fingerling chinook, the possibility is raised that immune competency was compromised by TBKME exposure. This possibility can be examined in light of studies using rainbow trout which showed that 2,3,7,8-TCDD injected intraperitoneally (10 $\mu g \cdot kg^{-1}$) suppressed immune responses (Spitsbergen et al. 1986). Immune responses remained normal among rainbow trout that received 2,3,7,8-TCDD doses equal to 0.1 and 1.0 $\mu g \cdot kg^{-1}$. The latter dose had a TEQ of 1000 $ng \cdot kg^{-1}$ which is more than 400 times the highest TEQ measured in fingerling chinook (Table 3). This comparison suggests that the TEQ body burdens measured among TBKME-exposed chinook (Table 3) were unlikely to have suppressed immune responses. Thus, if immune response was depressed by laboratory TBKME exposure, it appears that the causative agents would have been something other than dioxins and furans. We recommend field studies to examine whether TBKME exposure causes immune suppression among wild fish.

IRND's increased during 60- and 144-d exposures to TBKME compared with controls (Table 5). An increase in IRND is considered to be a response to chronic stress, such as exposure to dehydroabietic acid, a component of BKME, and is usually detectable in 24–48 h (Donaldson et al. 1984). Our data suggest that the IRND stress response threshold is about 1.5% (v/v) TBKME at 60 d of exposure and about 0.8% (v/v) at 144 d. On the other hand, there was no IRND response among juvenile coho (*Oncorhynchus kisutch*) exposed 25 d to one-third the 96 h LC50 of pH-neutralized BKME (McLeay 1973). There appears to be no ready explanation for the different IRND observations reported in McLeay (1973) and our results.

An increase in IRND is not limited to the chronic stresses caused by toxicants. We suspect that the high IRND values among control fish at 60 d, compared with the other time intervals, indicate that low temperature (0.9°C) during the 60-d period imposed a stress. This result is consistent with a study of fingerling coho in which IRND's enlarged owing to exposure to 2°C for 14 d (Allen 1971). In our study, TBKME-exposed fish exhibited an even greater IRND response than controls at 60 d. We believe this is evidence of a combined stress exerted by environmental factors (low temperature) and toxic chemical exposure (TBKME).

EROD activity was elevated 2.4–3.0 times relative to control values during exposure to TBKME (Fig. 4). Dioxins and furans are known to induce EROD activity, but there are no established relationships between EROD levels and TEQ body burdens for EROD induction in salmonids. To test for a relationship between EROD activity and TEQ, we examined two correlations using our laboratory data and field results from other sources. The first correlation was between relative EROD and TEQ and the second between EROD and TEQ. TEQ values were based on relative acute mortalities (Walker and Peterson 1991). In the first method, EROD data were converted to relative values based on the mean control EROD at the time of sampling. This procedure takes account of the fact that control EROD activity varied between sampling times as seen in Fig. 4. EROD levels were elevated above reference values in fingerling chinook salmon collected from the Fraser River at two sites downstream of bleached kraft pulp mills in 1988 (Rogers et al. 1989) and at four sites in 1989 (I.H. Rogers, Institute of Ocean Sciences, Sydney, B.C., Canada V8L 4B2, unpublished data).

The methodology was the same in the 1988 and 1989 studies. Relative EROD for field data were calculated based on corresponding EROD measurements at the reference sites. The TEQ and relative EROD data from laboratory and field measurements are plotted in Fig. 7. We calculated ANOVA (GLM) for all the data and obtained $p = 0.0001$, confirming that significant differences exist between means. Regression yielded equation 2 ($p < 0.01$, $r = 0.843$, df = 270):

$$(2) \qquad y = 0.906 + 0.695x$$

where y is relative EROD and x is TEQ (nanograms per kilogram).

In the second method of testing for a relationship between EROD and TEQ, all the laboratory and field TEQ and EROD data (not relative EROD) were subjected to ANOVA (GLM) for which $p = 0.0001$. Regression of these data produced equation 3 ($p < 0.01$, $r = 0.728$, df = 270):

$$(3) \qquad y = 0.015 + 0.003x$$

where y is EROD (nanomoles per milligram per minute) and x is TEQ (nanograms – per kilgram). The higher value of r for equation 2 indicates that relative EROD and TEQ have the better correlation.

Although equation 2 is linear, we have chosen a logarithmic plot to spread the data at low values of TEQ because this helps identify a possible threshold for EROD induction. At 0.3 TEQ and less, there are seven data points at 1.0 mean relative EROD. To the right of 1.0 TEQ, all the mean relative EROD data points equal or exceed 1.47, with the exception of one. (The exception represents control group C at 60 d where the TEQ would be 0.22 $ng \cdot kg^{-1}$ were it not for a 2,3,7,8-TCDD measurement at the MDC (Table 3).) These data suggest an approximate threshold for EROD induction between 0.3 and 1.0 ng $TEQ \cdot kg^{-1}$ where TEQ is based on relative dioxin/furan acute mortality. This correlation does not prove that TCDD and TCDF were the only inducing compounds in either the field or laboratory data, but if these were not the only inducers, then the unidentified inducers appear to correlate with them.

In summary, this study indicates that laboratory exposure of fingerling chinook salmon to nonlethal TBKME at environmentally realistic concentrations and temperatures did not compromise freshwater growth, seawater acclimation, or subsequent growth in seawater. Laboratory exposure led to contaminant burdens, but the low body burdens of dioxins and furans compared with wild fingerling chinook indicated that the laboratory environment did not account for bioconcentration of these substances via the food chain. However, the study supported previously published results relating chlorophenol body burdens and aqueous exposure concentrations. Analysis of laboratory and field data yielded a relationship between dioxin TEQ and EROD induction. EROD induction was the most dramatic response among the 13 parameters measured, but the implications of EROD induction for survival of wild fish have not been defined. Histopathology indicated that TBKME-exposed fish experienced BKD and were subjected to chronic stress as seen in IRND measurements. The linkage between disease and TBKME exposure was tenuous.

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Unexpected Changes in Reproductive Rates and Mean Age at First Birth during the Decline of the Pribilof Northern Fur Seal (*Callorhinus ursinus*)

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Trites, A.W., and A.E. York. 1993. Unexpected changes in reproductive rates and mean age at first birth during the decline of the Pribilof northern fur seal (*Callorhinus ursinus*). Can. J. Fish. Aquat. Sci. 50: 858–864.

From 1956 to 1968, female northern fur seals (*Callorhinus ursinus*) were harvested on the Pribilof Islands, Alaska, in an effort to increase the productivity of the herd. In theory, pregnancy rates should have increased and the age at first birth should have declined as population density was reduced. Instead the opposite happened: pregnancy rates dropped and age of first birth increased. It is unlikely that these changes were caused by shortages of food or poor physical condition of the females, given that body size increased over this period. The most likely explanations for the changes observed between 1958 and 1974 are related to altered age and sex ratios of breeding animals caused by the depletion of females and/or the harvesting of young males. Changes in pregnancy rates and age at first birth are inconsistent with the density-dependence paradigm and suggest that relative densities of mature age and sex classes on the breeding beaches (a product of social interactions and territory size) may be more consequential than absolute population densities in affecting the reproductive biology of northern fur seals.

De 1956 à 1968, des femelles d'otarie à fourrure (*Callorhinus ursinus*) ont été récoltées dans les îles Pribilof (Alaska) afin d'accroître la productivité du troupeau. En théorie, le taux de gravidité aurait dû augmenter et l'âge de la première mise bas aurait dû diminuer suite à cette baisse de la densité de la population. Au contraire, les effets opposés ont été notés : le taux de gravidité a diminué et l'âge à la première mise bas a augmenté. Il est peu probable que ces modifications s'expliquent par un manque de nourriture ou une mauvaise condition physique des femelles, car la taille des individus a augmenté pendant cette période. L'explication la plus plausible des changements observés entre 1958 et 1974 a trait à une modification des rapports des âges et des sexes des reproducteurs découlant du manque de femelles ou de la récolte de jeunes mâles. Les modifications des taux de gravidité et de l'âge à la première mise bas ne concordent pas avec le modèle de la dépendance envers la densité. Cela porte à croire que, en ce qui a trait à la biologie de la reproduction chez les otaries à fourrure, les densités relatives des classes d'âge d'individus matures et des classes de sexes des animaux présents sur les plages de reproduction (qui découlent des interactions sociales et de la taille des territoires) sont plus importants que les densités de population absolues.

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The abundance of northern fur seals (*Callorhinus ursinus*) on the Pribilof Islands, Alaska, increased from 200 000 to 300 000 animals in 1911 to over 1.5 million in the late 1940s under a management policy that protected females but allowed the harvesting of immature males (Kenyon et al. 1954; Lander and Kajimura 1982). When population growth slowed during the 1940s, it was suggested that the herd's productivity had been lowered by reduced rates of reproduction (Kenyon et al. 1954) and juvenile survival (Chapman 1961) arising from competition for a limited food supply around the Pribilof Islands. Mathematical models subsequently suggested that greater productivity (and a larger harvest) could be achieved from a smaller population (Chapman 1961; Nagasaki 1961). As a result, 315 000 females were killed between 1956 and 1968 in addition to the continued male harvest (Lander 1980a). However, annual pup production and recruitment of harvestable males failed to increase as predicted, and the population declined (Fig. 1; Chapman 1981).

Lowering the herd size was supposed to increase productivity through density-dependent changes in population vital rates. In particular, reproductive rates should have increased and mean age at first birth should have decreased. Whether or not these changes occurred is the subject of our study.

From 1958 to 1974, over 11 000 female fur seals were collected at sea by Canada and the United States as part of a pelagic research program (Lander 1980b). Based on macroscopic observations of reproductive tracts (Lander 1980b), females were classified as nulliparous (female has never been impregnated), primiparous (pregnant only once, regardless of whether the pregnancy is carried to term), and multiparous (previously

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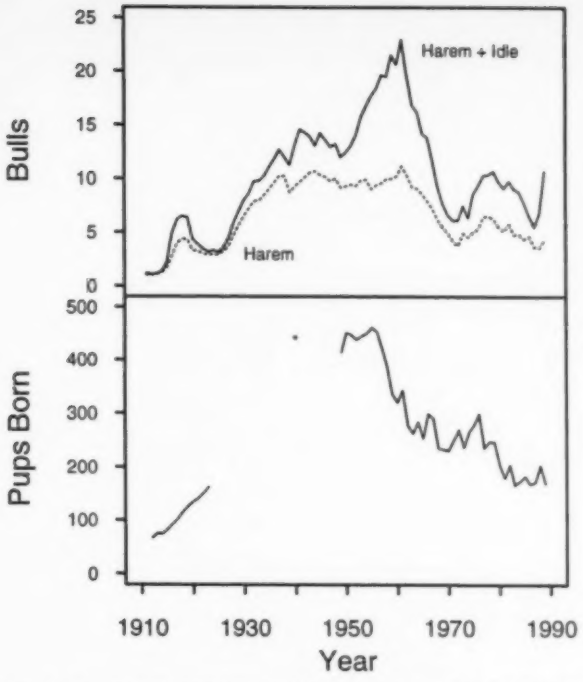

FIG. 1. Numbers of pups born and bulls counted on St. Paul Island (one of the Pribilof Islands) from 1911 to 1989 (in thousands). The data are an index of population abundance and are taken from Lander (1980a), York (1985), and Trites (1989).

pregnant more than once, and either pregnant or nonpregnant at the time of collection). The pelagic samples were all collected in the North Pacific Ocean from California to the Bering Sea, with month and region of sampling varying between years.

Our study used the pelagic data base to test whether reproductive rates increased and age at first birth decreased as the population declined. We estimated annual pregnancy rates and examined the frequency of multiparity among the females sampled. We also estimated the mean age when females gave birth for the first time from 1959 to 1974. Finally we explored the relationship between body size and reproductive condition and considered the influence of fur seal density on reproductive rates.

Pregnancy Rates

Early analysis of changes in pregnancy rates in the 1950s showed no appreciable change with time (Nagasaki 1961; Chapman 1964). Further analysis of additional pelagic data by Smith and Polacheck (1984) also indicated little change in pregnancy rates over the years 1958–74. However, the authors did not control for geographic effects and other possible sources of sampling bias. Additional analysis by Bigg (1979a) and York (1979) found considerable seasonal and regional variation in pregnancy rates associated with the migratory patterns of females of different ages and reproductive conditions. The highest pregnancy rates were recorded near the breeding beaches during the summer pupping period. Pregnancy rates were lower farther south along the Aleutian chain, while still lower rates were calculated from samples taken in the coastal waters of Washington during winter and spring. In the Gulf of

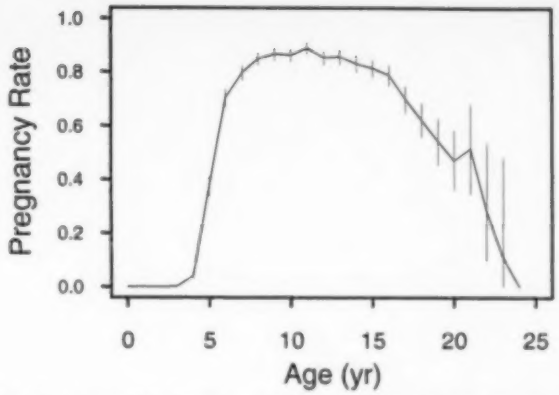

FIG. 2. Age-specific pregnancy rates (including postpartum animals) of 15 020 seals collected at sea in all sampling areas from 1958 to 1974. The vertical bars are 95% confidence intervals. Data from Lander (1981).

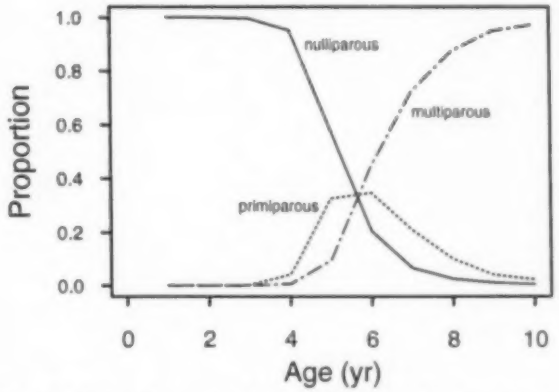

FIG. 3. Age-specific reproductive condition of 10 824 females ages 0–10 yr collected at sea in all sampling areas from 1958 to 1974.

Alaska, the pregnancy rate for sampled females rose and fell as the females migrated northward during late spring/early summer to give birth on the Pribilof Islands.

Pregnant females return to the Pribilofs about 3–4 wk earlier than nonpregnant females (Bigg 1990; Trites 1990). Pregnant and nonpregnant females appear to be more or less randomly mixed from December to March while in the coastal waters of California, Oregon, Washington, and British Columbia (Bigg 1990). They also appear to be randomly mixed from July to September around the Pribilof Islands (55–59°N, 166–174°W). We therefore estimated pregnancy rates for these two regions during the respective time periods. We did not include samples taken from April to June because females were segregated at this time by age and reproductive condition as they migrated northward through the Gulf of Alaska and on by the Aleutian chain.

Females begin bearing young as early as age 4 yr (see Fig. 2 and 3). The pregnancy rate rises rapidly until age 8 whereupon it remains relatively constant for the next 5 yr. After age 14, the pregnancy rate declines each year until the seal completely stops reproducing when about 25 yr old.

Erroneous estimates of pregnancy rates can result from biases hidden in the data (York 1980). For example, 6% (5 of 85) of

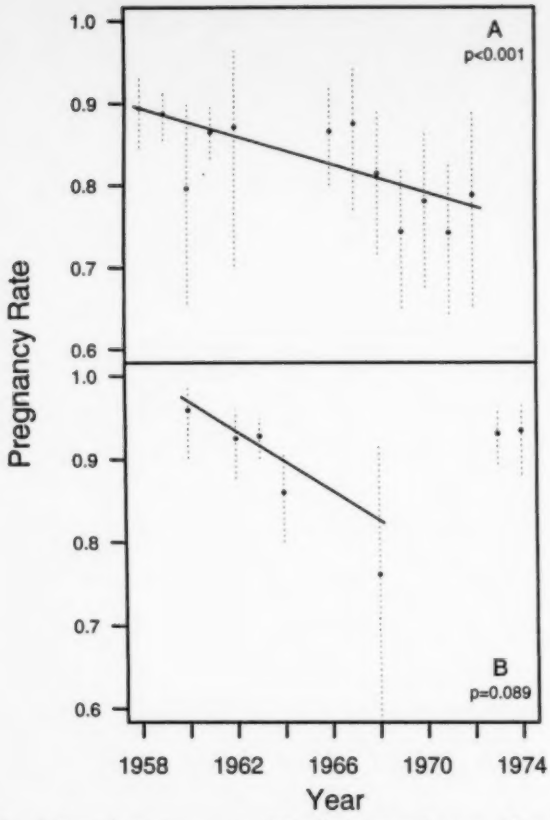

FIG. 4. Annual pregnancy rates of females between the ages of 8 and 13 yr collected from (A) California to British Columbia and (B) in the Pribilof region. The data were fit with weighted linear least squares regressions. Broken lines are 95% confidence limits.

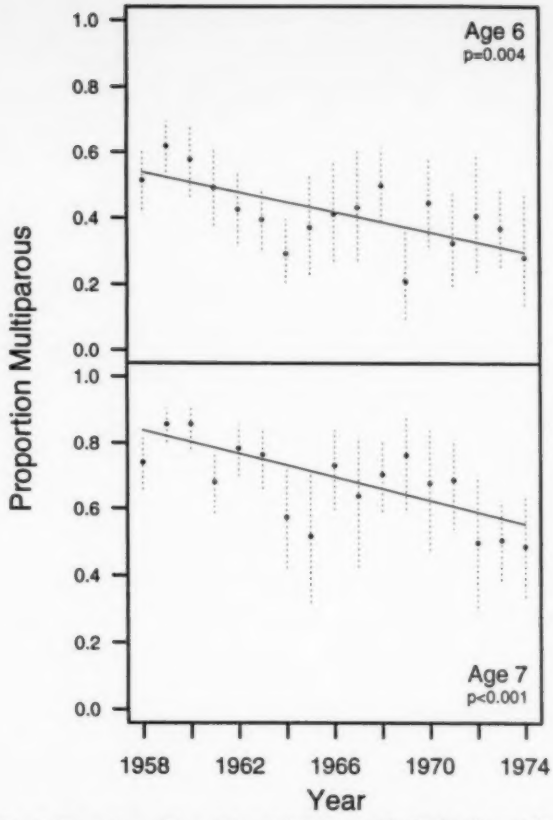

FIG. 5. Proportion of 6- and 7-yr-old females collected in all areas that were multiparous in the 1958–74 pelagic samples. The significance of weighted linear regressions is shown in the panels. Broken lines are 95% confidence limits.

the 4-yr-old females from the 1954 year class were primiparous, implying that the pregnancy rate for 3-yr-olds was 6%. At age 5, 8% (11 of 140) of this year class were primiparous, but not pregnant when captured, and 19% (27 of 140) were multiparous. Thus the pregnancy rate of 3-yr-olds must have been between 19 and 27%, substantially higher than the 6% directly observed. This discrepancy might simply be due to binomial sampling variance. On the other hand, the discrepancy in estimated rates of pregnancy in 3-yr-olds might reflect differential survival rates for pregnant and nonpregnant females or could have arisen if mature females were easier to capture than immature females. Estimates of reproductive rates could also be easily biased by changes in age structure during the intervening time. Hence, we only estimated pregnancy rates for females between the ages of 8 and 13 yr because pregnancy rates vary little for this age group (York 1979; Lander 1981) and because some models for mammalian survivorship (e.g., Siler 1979) suggest that mortality rates should be relatively age constant over this age range (8–13 yr). Furthermore, northern fur seals are at their reproductive prime between 8 and 13 yr based on the size of the fetuses they carry (Trites 1991).

Pregnancy rates of 8- to 13-yr-old females collected in the Pribilof and California – British Columbia regions declined from 1958 to 1972 (Fig. 4; cf. Eberhardt 1990). Weighted linear least squares regressions, with weight inversely proportional to the variance, indicate a highly significant drop in pregnancy rates for the large California – British Columbia samples ($r^2 = 0.688$, $F_{1,10} = 22.03$, $p < 0.001$). Pregnancy rates of animals sampled in the Pribilof region were higher than the southern sample and also suggest a decline in pregnancy rates from 1960 to 1968 ($r^2 = 0.673$, $F_{1,3} = 6.16$, $p = 0.089$). Pregnancy rates may have risen abruptly after 1968 (Fig. 4B), although these high estimates may simply be artifacts of sampling too close to the rookeries in 1973 and 1974 (York 1979; Smith and Polacheck 1984).

Proportion of Multiparous Females

Few females have had two or more pregnancies by the age of 5 (Fig. 3). But by the ages of 6 and 7, the proportion of multiparous females is large, until virtually all females are multiparous at age 8.

The conclusion that pregnancy rates declined as the population dropped is supported by the changing proportion of multiparous females captured. The proportion of 6- and 7-yr-old females that were multiparous in the 1958–74 pelagic samples (Fig. 5) declined with time (weighted linear regressions – age 6: $r^2 = 0.4405$, $F_{1,15} = 11.81$, $p = 0.004$; age 7: $r^2 = 0.5867$, $F_{1,15} = 21.29$, $p < 0.001$). This suggests that pregnancy rates must have dropped and/or that the age of females at first estrus increased as the population declined.

TABLE 1. Number of female northern fur seals captured in 1959 and 1960 that were nulliparous, primiparous, and multiparous and the proportion that were currently or previously pregnant $p_{a,t}$.

	1959				1960			
Age	nulli	primi	multi	$p_{a,t}$	nulli	primi	multi	$p_{a,t}$
1	79	0	0	0.00	26	0	0	0.00
2	55	0	0	0.00	63	0	0	0.00
3	46	1	0	0.02	72	0	1	0.01
4	101	14	1	0.13	50	3	1	0.07
5	45	68	27	0.68	32	33	20	0.62
6	14	46	98	0.91	12	23	48	0.86
7	5	22	165	0.97	2	14	97	0.98
8	1	14	193	0.97	3	11	139	0.98
9	0	5	138	1.00	0	4	177	1.00
10	0	1	116	1.00	0	4	159	1.00

The proportion of females estimated to be multiparous represents all regions of the North Pacific and all months. The likelihood of biased estimates arising from segregated migrations was reduced because samples were from a wide geographical and temporal range. Furthermore, while females may segregate on the basis of age and whether they are pregnant or nonpregnant, there is no evidence that they segregate on the basis of parity (Bigg 1990; Trites 1990). Hence, we felt it was appropriate to pool the data.

Age at First Birth

Early analysis of pelagic data suggested that the age at first birth changed in a density-dependent fashion (Nagasaki 1961; Chapman 1964). Subsequently, York (1983) calculated the average age at first birth for individual year classes (1952–66) and found that it abruptly increased beginning with the 1956 year class. Age at first birth subsequently decreased but never to the level of the 1952–55 year classes. Possible explanations offered were an impact on prey abundance by commercial fishing or a selection during the female harvest towards taking females that matured earlier.

In the current analysis, we modified the methods of DeMaster (1978, 1981) and York (1983) to estimate the mean age at first birth. We calculated the females' mean age at first birth based on the year of collection rather than on the year class as has been done previously. We took this approach because the attainment of sexual maturity is more likely to be a function of annual feeding conditions than accumulated cohort growth (see Discussion).

Mean age at first birth in any given year is estimated as follows. The proportion of females that are pregnant by age a in year t is

$$(1)\quad p_{a,t} = \frac{n_{a,t}}{N_{a,t}}$$

where $n_{a,t}$ is the number of females in the sample that have ovulated at least once in their lifetime (primiparous + multiparous females) and $N_{a,t}$ is the total number of females in the sample (nulliparous + primiparous + multiparous females). The probability of being pregnant for the first time at age a in year t is

$$(2)\quad \hat{p}_{a,t} = p_{a,t} - p_{a-1,t-1}$$

where $\hat{p}_{a,t} \geq 0$. From this, the average age at first birth $\bar{A}$ in year t is

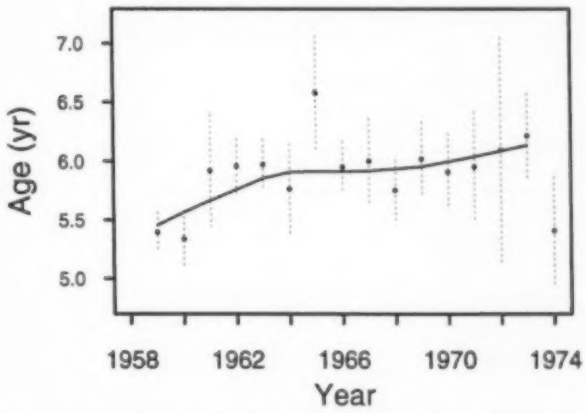

FIG. 6. Mean age at first birth in 1959–74. The trend is shown with a locally weighted robust regression (lowess, $f = 0.6$). Broken lines are 95% confidence limits.

$$(3)\quad \bar{A}_t = \frac{\sum_{a=1}^{w} a\,\hat{p}_{a,t}}{\sum_{a=1}^{w} \hat{p}_{a,t}}.$$

To illustrate using the data in Table 1 for the females sampled in 1960: $\sum_{a=1}^{10} \hat{p}_{a,t} = 0.00 + 0.00 + 0.01 + 0.05 + 0.49 + 0.18 + 0.07 + 0.01 + 0.00 + 0.00$ and $\sum_{a=1}^{10} a\hat{p}_{a,t} = 0.00 + 0.00 + 0.04 + 0.21 + 2.47 + 1.06 + 0.50 + 0.05 + 0.04 + 0.00$. Thus the mean age at first birth in 1960, $\bar{A}_{1960}$, was 5.34 yr.

The variance of $\bar{A}_t$ is estimated with the jackknife procedure (Mosteller and Tukey 1977). Based on k sample observations ($k = \sum_{a=0}^{w}[N_{a,t} + N_{a-1,t-1}]$), k estimates of $\bar{A}_t$ are calculated, each time omitting one sample observation. Next, k pseudo-values $J_{i,t}$ are calculated, utilizing the mean age of the full sample $\bar{A}_t$ and the mean age $\bar{A}_{i,t}$ obtained by omitting observation i:

$$(4)\quad J_{i,t} = k\bar{A}_t - (k-1)\bar{A}_{i,t} \qquad i = 1, 2, \ldots, k.$$

The variance of the k pseudo-values $J_1, J_2, \ldots, J_k$ is

$$(5)\quad \mathrm{Var}(\bar{A}_t) = \frac{\sum_{i=1}^{k} J^2_{i,t} - \frac{1}{k}\left(\sum_{i=1}^{k} J_{i,t}\right)^2}{k(k-1)}.$$

Degrees of freedom are equal to the number of different pseudo-values minus 1.

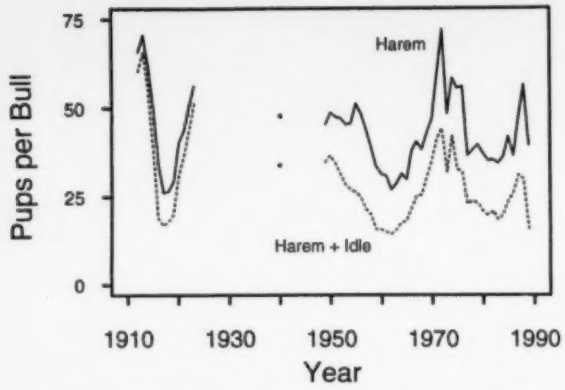

FIG. 7. Ratio of pups born to the number of bulls counted on St. Paul Island. The solid line is a rough measure of harem size based on data from Fig. 1. The broken line shows the ratio of females giving birth to the total number of mature bulls present (harem bulls + idle bulls). Harem bulls actively defend territories with one or more females. Idle bulls are also full grown and defend a territory, but have no females.

The mean age of females giving birth for the first time, as calculated with the above procedure, rose from 1959 and 1974 (Fig. 6). A weighted linear regression, with weight inversely proportional to the variance, indicates that this rise is significant ($r = 0.56, F_{1,14} = 6.41, p = 0.02$). The anticipated decline in age at first birth in response to the population reduction did not occur.

Discussion

A female's ability to conceive is likely related to food availability and body fat. Many pinniped studies have certainly recognized the importance of growth and nutrition in the attainment of sexual maturity (Laws 1956, 1959; Bryden 1968; Sergeant 1973, 1978; Bowen et al. 1981; Innes et al. 1981; Lett et al. 1981). However, none has considered the critical importance of body fat in relation to body size. Studies of rats and pigs demonstrate that individuals given a high fat diet have first estrus significantly earlier than others fed a low fat diet of equal calories (Kennedy and Mitra 1963; Dickerson et al. 1964; Frisch et al. 1975). Human studies further support the view that females must store at least a threshold or minimum amount of body fat to begin and maintain normal menstrual cycles and hence have the ability to reproduce (Frisch and McArthur 1974; Frisch 1978, 1985).

Nulliparous fur seals ovulate for the first time in August and September (Craig 1964). Based on the studies of rats, pigs, and humans, the weight of the seal in relation to her length probably governs whether or not she ovulates. Thus, high pregnancy rates in one year should correspond to a high ratio of body weight to length the previous year. We attempted to test this by correlating pregnancy rates with body mass of females collected near the Pribilofs from June to August of the previous year. Unfortunately the data were too sparse and no comparison could be made. However, it appears from growth curves that northern fur seals attained larger body sizes as the population declined through the 1960s (Bigg 1979b; Trites and Bigg 1992). Thus, it seems unlikely that the drop in pregnancy rates could be related to body size, given that pregnancy rates dropped as body size increased. Note, however, that there may be a relationship between the annual growth rate of immature females (fig. 4 in Trites and Bigg 1992) and the proportion of multiparous 6-yr-olds (Fig. 5). These data suggest comparable periodic changes from 1958 to 1974 in both growth rates and the proportion of females that were multiparous.

Another factor that might influence pregnancy rates is a shortage of bulls to service the females (Kenyon et al. 1954; Scheffer 1955). A rough measure of harem size and hence the availability of males is the ratio of pups born to the number of bulls counted (calculated from Fig. 1). As shown in Fig. 7, the average harem size (which excludes harem females without pups) has fluctuated between 25 and 75 females per bull since 1911. Much of this variation is probably associated with the commercial harvesting of males and females. As such, it is difficult to determine what the natural or optimum ratio of males to females is and how this ratio might affect reproductive rates. What is clear, however, is that the harem size increased rapidly from 26.8 females per male in 1962 to 72.0 females per male in 1972. At the same time, pregnancy rates declined (Fig. 4A).

The correlation between harem size and pregnancy rates (ages 8–13 yr, California – British Columbia) is significant (weighted linear regression: $r^2 = 0.370, F_{1,10} = 5.87, p = 0.036$) as is the relationship between pregnancy rates and females per harem plus idle bulls ($r^2 = 0.602, F_{1,10} = 15.11, p = 0.003$). However, correlations are weaker if pregnancy rates are lagged by 1 yr (harem: $r^2 = 0.039, F_{1,10} = 0.40, p = 0.539$; harem + idle: $r^2 = 0.380, F_{1,10} = 6.14, p = 0.033$). This raises two points. First, idle bulls may have a major impact on pregnancy rates. Second, caution needs to be exerted in interpreting these correlations because both sets of variables contain errors and represent parameter changes in a single direction. More conclusive statements could be made only if pregnancy rates had been estimated through the 1970s as the harem size decreased.

Gentry and Goebel (1982) observed copulations on the breeding beaches and did manipulative experiments with captive males and females to study reproductive behaviour. They found that females enter estrus rapidly and spontaneously between 5 and 7 d postpartum but can be induced by males (cf. Craig 1964). Estrus will only last up to 34 h if the female fails to copulate and will not reoccur later in the breeding season. However, estrus can be terminated within a few minutes of copulation depending upon the diameter of the terminal bulb of the penis and the pattern of pelvic thrusting. Thus, while females are receptive to males ranging in size from 45 to 135 kg (age 5 yr and older, based on growth curves in Trites 1990), the smaller males are not as effective at terminating estrus. Nevertheless, the young males are fertile and successfully impregnate females. This suggests that altering the sex ratio by harvesting immature males could affect the overall rate of reproduction if young males are not available in sufficient number to mate with late arriving young females (Fowler 1990).

The period from the late 1950s to early 1970s was probably one of great turmoil on the rookeries. On one hand, there was a sharp rise and then drop in the numbers of idle bulls seeking females (Fig. 1). On the other, some of the harems were disturbed by rounding up females from the rookeries for harvesting, while others became depleted over time as females harvested from hauling grounds failed to return to their pups (Roppel 1984; Scheffer et al. 1984). The size of the female harvest was large and occurred over a brief period of time (1956–68). Indeed, killing mature females can account for over 70% of the drop in numbers of pups born through the 1960s

(Fig. 1; York and Hartley 1981; Trites and Larkin 1989). All told, these changes during the period of harvesting males and females undoubtedly altered social structure and interaction on the rookeries. This in turn could have lowered pregnancy rates by reducing social stimulus needed to successfully mate or by disrupting mating during the few hours of estrus.

The changes observed from 1958 to 1974 in pregnancy rates and age at first birth were not the anticipated density-dependent responses. Instead of rising, productivity dropped during the years of herd reduction. The most likely explanation for this unexpected result is that reproduction was a function of relative densities of mature age and sex classes in harems rather than absolute population density. The decision to harvest females was based on theoretical concepts of density dependence which have changed little over the past three decades. The failure of the female harvest to achieve its goals contributed to the decline of the herd and demonstrates our incomplete understanding of fur seal population biology. Insights gained from this shortcoming suggest that greater emphasis should be placed upon rookery social structure and fur seal reproductive behaviour in future efforts to manage the northern herds.

Acknowledgements

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Whole-Lake Burdens and Spatial Distribution of Mercury in Surficial Sediments in Wisconsin Seepage Lakes

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We quantified total mercury in surficial sediments (uppermost 5 cm) of six small seepage lakes. Fifty cores were taken from each lake, based on a random sampling design stratified by water depth. Volumetric concentrations (mass per volume of wet sediment) more accurately portrayed the depth distribution of mercury in the lakes than did dry-weight concentrations, which underrepresented the significance of the shallow-water sediments as a reservoir of potentially available mercury. Estimates of whole-lake burdens (masses) of mercury in the surficial sediment, which represent the maximum amount of sedimentary mercury available for methylation, ranged from 45 to 149 g. These sedimentary pools of mercury greatly exceeded the inventories of the metal in water, seston, and fish, and the release of mercury from the sediments could significantly increase its bioaccumulation. Areal burdens of mercury (mass per unit of lake area) varied fivefold among lakes (1.65–7.84 g/ha) and were strongly correlated with lake pH, but were not correlated with dissolved organic carbon, conductance, or chlorophyll *a* of lakes. We hypothesize that the observed variation in areal burdens of mercury was partly due to variation among lakes in the pH-related efflux of gaseous mercury (Hg^0) from the lakes to the atmosphere.

Nous avons mesuré le mercure total dans des sédiments superficiels (couche supérieure de 5 cm) provenant de six petits lacs d'infiltration. Cinquante carottes ont été prélevées dans chacun des lacs, d'après un plan d'échantillonnage aléatoire stratifié en fonction de la profondeur de l'eau. Les concentrations volumétriques (masse par volume de sédiments humides) ont représenté plus fidèlement la distribution du mercure dans les lacs en fonction de la profondeur que les concentrations en poids sec, qui ont eu tendance à sous-représenter l'importance des sédiments en eau peu profonde comme réservoir de mercure potentiellement disponible. L'estimation pour les lacs entiers des charges (masse) de mercure dans les sédiments superficiels, qui représente la quantité maximale de mercure d'origine sédimentaire disponible pour la méthylation, a varié de 45 à 149 g. Ces réservoirs de mercure sédimentaire dépassent largement les inventaires de ce métal dans l'eau, le seston et le poisson, et la libération du mercure présent dans ces sédiments pourrait augmenter substantiellement sa bioaccumulation. Les charges surfaciques de mercure (masse par unité de surface de lac) ont varié dans un rapport de 1 à 5 selon les lacs (de 1,65 à 7,84 g/ha) et avaient une forte corrélation avec le pH du lac, mais non avec la carbone organique dissous, la conductance ou la chlorophylle *a*. Nous avons formulé l'hypothèse que la variation observée dans les charges surfaciques de mercure était en partie attribuable à la variation observée entre les lacs en ce qui a trait à la vaporisation du mercure gazeux (Hg^0) liée au pH, du lac vers l'atmosphère.

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Much of the mercury that enters freshwater lakes is deposited in bottom sediments (Fitzgerald et al. 1991), which are widely considered to be a sink for the metal. The sediments also represent a large reservoir of potentially bioavailable mercury, although the availability of sedimentary mercury for methylation and subsequent biological uptake is little understood (Gilmour and Henry 1991). The widespread enrichment of surficial lacustrine sediments with mercury in the Northern Hemisphere is widely interpreted as evidence of recent anthropogenic releases of the metal into the atmosphere (Evans 1986; Rada et al. 1989; Verta et al. 1989; Norton et al. 1990; Swain et al. 1992). Slemr and Langer (1992) reported that atmospheric concentrations of total gaseous mercury in the Northern Hemisphere increased about 1.5%/yr during 1977–90, presumably as a result of anthropogenic releases.

Studies of mercury accumulation by freshwater fish indicate that within-lake processes can significantly influence the bioaccumulation of the metal (Wiener et al. 1990a, 1990b). In low-pH lakes the bioavailability of mercury is enhanced (Spry and Wiener 1991), due in part to the greater microbial production of methylmercury (the form most readily accumulated in fish) at low pH (Xun et al. 1987; Miskimmin et al. 1992). The microbial production of methylmercury is most rapid near the sediment–water interface and seems to be increased by acidification (Xun et al. 1987; Winfrey and Rudd 1990; Gilmour and Henry 1991; Miskimmin et al. 1992).

As part of a multidisciplinary study of the biogeochemistry of mercury in temperate lakes (Fitzgerald et al. 1991), we

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TABLE 1. Characteristics of the study lakes in north-central Wisconsin, listed in order of increasing mean pH. Water chemistry data are mean epilimnetic values for 1990 (from Watras et al. 1991) for all lakes except Max Lake.[a] ANC is acid-neutralizing capacity, DOC is dissolved organic carbon, and K_s is specific conductance.

Lake	Coordinates	pH	ANC (μeq/L)	DOC (mg/L)	K_s (μS/cm)	Surface area (ha)	Depth (m)	
							Maximum	Mean
Max	46°01′N, 89°42′W	5.16	−5	2.1	16.0	9.2	6.1	2.9
Russett	46°12′N, 89°36′W	5.77	27	6.8	15.1	19.0	11.0	2.9
Little Rock[b]	46°00′N, 89°42′W	6.12	25	3.0	13.5	8.1	6.5	3.1
Crystal	46°00′N, 89°37′W	6.34	25	1.7	13.6	36.0	20.4	10.4
Vandercook	45°59′N, 89°40′W	6.37	42	4.1	16.2	43.6	7.2	4.7
Pallette	46°04′N, 89°36′W	7.03	127	5.3	22.8	70.0	18.2	9.6

[a]The chemistry of Max Lake was experimentally altered by pumping groundwater into the basin, beginning in April 1990. Chemical data shown for Max Lake are from 1988–89, before manipulation of the lake's chemistry (P.J. Garrison, Wisconsin Department of Natural Resources, Madison, WI, personal communication).

[b]Reference basin only.

examined the spatial distribution and burdens of mercury in the surficial sediments of seepage lakes in north-central Wisconsin. In this paper, we (1) describe the spatial distribution of mercury in surficial sediments of six Wisconsin seepage lakes, (2) present estimates of the whole-lake burdens of mercury in the surficial sediments, and (3) examine the relation of the areal burdens of mercury to selected physical and chemical characteristics of the lakes. The term "whole-lake burden," as applied in this paper, refers to the total mass of mercury in the uppermost 5 cm of sediment in an entire lake. The term "areal burden" refers to the mean mass of mercury in the uppermost 5 cm of sediment per unit of area in a lake.

Methods

Study Area

The six study lakes are in north-central Wisconsin (Vilas County) in the Northern Highland Lake District, an area with thousands of lakes formed during the Pleistocene glaciation. About 40% of the lakes in the district have low acid-neutralizing capacity (ANC < 50 μeq/L), and about 3% are acidic (ANC ≤ 0; Eilers et al. 1988). The study lakes are small (8–70 ha) seepage basins (no surface inlets or outlets) and span a broad range of pH (5.2–7.0), ANC (−5–127 μeq/L), and dissolved organic carbon concentrations (DOC, 1.7–6.8 mg/L; Table 1). Further description of the area and study lakes has been given elsewhere (Eilers et al. 1983; Wiener and Eilers 1987; Watras et al. 1991).

The low-ANC seepage lakes in this study have recently received nearly all (> 95%) of their hydrologic inflow from precipitation directly onto the lakes (Krabbenhoft and Babiarz 1992). Only Pallette and Russett lakes received measurable groundwater input during 1987–90 (Krabbenhoft and Babiarz 1992). Hydrologic residence times are long in these seepage lakes, about 4–5 yr in Vandercook Lake, for example (Schnoor et al. 1986). The lakes are usually ice covered from mid-November until early April.

The lakes are in rural, mostly forested watersheds with no identifiable, on-site anthropogenic sources of mercury and no mercury-enriched ores. Yet, mercury concentrations are high in fish from low-pH lakes in the district (Cope et al. 1990; Grieb et al. 1990; Wiener et al. 1990b), commonly exceeding criteria for issuance of fish-consumption advisories (Wisconsin Department of Natural Resources and Wisconsin Division of Health 1991). Moreover, surficial sediments in these lakes are mercury enriched, relative to deeper, precolonial sediments (Rada et al. 1989; Swain et al. 1992). Annual atmospheric inputs of mercury to these lakes (wet plus dry deposition) have averaged about 0.1 g/ha during 1988–90 (Fitzgerald et al. 1991); this atmospheric influx is sufficient to account for the mass of mercury accumulating in fish and sediment of Little Rock Lake (Wiener et al. 1990a; Fitzgerald et al. 1991).

Little Rock Lake, the site of an experimental acidification study, was separated in September 1984 with a plastic sea curtain into two experimental units, a treatment basin (area 9.8 ha; mean depth 3.9 m) and a reference basin (8.1 ha; 3.1 m). Experimental acidification of the treatment basin with sulfuric acid began in spring 1985 and continued for 6 yr (Watras and Frost 1989). In this paper, we present data only for the reference basin, which was not acidified.

Sampling Design and Field Methods

A random sampling design, stratified by water depth, was used to allocate the sampling effort spatially in each lake, with the following equation (adapted from Gilbert 1987):

$$(1)\quad N_i = N_t(W_i/\Sigma W_i)$$

where N_i = the number of sediment samples to be collected from the ith stratum within a lake (a stratum was the lake-bottom area between two depth contours on a morphometric map), N_t = the total number of sediment samples to be collected from the lake (defined a priori as $N_t = 50$), and W_i = the weight of the ith stratum, which was used to allocate sampling effort among strata, defined as

$$(2)\quad W_i = A_i \cdot D_i^{0.5}$$

where A_i = the area of water overlying the ith stratum and D_i = the approximate mean water depth (metres) of the ith stratum. The factor $D_i^{0.5}$ in equation 2 allocates more samples to sediment underlying deeper waters within each lake. Our decision to allocate greater sampling effort to deeper sediments was based on earlier data showing that dry-weight concentrations of mercury and volatile matter in surficial sediments from Little Rock Lake, one of our study sites, increased concomitantly with increasing water depth (Wiener et al. 1990a).

After N_i was established for each 1- to 4-m depth interval within a lake, sampling locations were randomly assigned on a morphometric map. The reference basin of Little Rock Lake was considered to be a whole lake in our experimental design.

Short sediment cores (uppermost 5 cm) were collected at Lit-

tle Rock (reference basin), Crystal, and Vandercook lakes during August 1988 and at Max, Pallette, and Russett lakes during June 1989. Sampling sites on each lake were located by triangulation. Many of the surficial sediments in the lakes were hydrous (>90% water) and flocculent; consequently, we used a large-diameter (20.3 cm inside diameter), open-barrel core sampler (modified from Rada et al. 1989) to reduce both resuspension of the surface floc and sediment compaction, two common problems of core sampling (Blomqvist 1991). The samplers were designed to enable the scuba diver operating the corer to isolate the uppermost 5 cm of sediment before removing the sampler from the sediments. Samples were placed in acid-washed plastic bags, held on ice in the dark in the field, and placed in a conventional freezer within 5 h after collection. Samples were stored at −20°C in the laboratory until processing (within 2–4 wk).

Just after inserting the large core sampler into the sediment, the diver took a second sample with a 50-cm^3, open-ended plastic syringe for the determination of the water content of sediment. The diver inserted the syringe barrel into the sediment while holding the syringe plunger at the sediment–water interface. While embedded in the sediment, the syringe barrel was sealed with a butyl rubber stopper to prevent leakage of sediment and pore water from the syringe. Samples were brought to the surface, placed in tared bottles, and sealed.

We consider the mercury in the uppermost 5 cm of the sediment profile to be a crude estimate of the maximum potentially bioavailable mass of mercury in physically undisturbed lake sediments. Potential rates of mercury methylation are greatest in this stratum and are negligible in deeper sediments (Rudd et al. 1983; Korthals and Winfrey 1987). Korthals and Winfrey (1987), for example, showed that ^{203}Hg added to littoral and profundal sediment cores from Clara Lake in northern Wisconsin was significantly methylated only to a depth of about 5 cm in the sediment profile. Moreover, a concurrent study in Little Rock Lake showed no significant diffusion of pore-water mercury to the water column from profundal sediments below the 2- to 4-cm stratum (Hurley et al. 1993).

Laboratory Methods

The syringe samples of sediment were thawed and analyzed for percent water (American Public Health Association et al. 1989). The sediment samples to be analyzed for total mercury and volatile matter were thawed, dried at room temperature, ground with a porcelain mortar and pestle, passed through a 2-mm sieve to remove coarse particles, and homogenized. Total volatile matter (an estimate of organic matter) was determined by loss on sample ignition at 550 ± 50°C for 1 h (American Public Health Association et al. 1989).

Total mercury determinations were made on subsamples (0.5–6.0 g, depending on the amount of volatile matter) that were digested with 15 mL of a 4:1 (vol:vol) solution of 18 M H_2SO_4 and 16 M HNO_3 (method modified from Ontario Ministry of the Environment 1988) in ignition tubes that were heated at 220°C for 14 h in aluminum blocks. After cooling, saturated aqueous $KMnO_4$ was added to the ignition tubes until a deep purple color persisted, and the digestates were further heated for 1 h at 130°C. After cooling and just before analysis, each digestate was transferred to a 300-mL Fleaker® and diluted to 100 mL with deionized water. Ten millilitres of a hydroxylamine solution (12% weight:vol) was added to the Fleaker®, and 5 mL of a 12% $SnCl_2$ solution was used as the final reductant. Each digestate was analyzed with an Instrumentation Laboratory 551 atomic absorption spectrophotometer equipped with an AVA 440 cold vapor generator.

Quality Assurance

Core samplers, sample containers, and laboratory glassware were acid-washed and rinsed with deionized water. Reagents used in digestions and analyses were suitable for use in mercury analyses.

To estimate the accuracy of our mercury determinations, we digested and analyzed the following with each analytical batch of samples: procedural blanks, calibration standards, procedural spiked samples, and National Institute of Standards and Technology (NIST) standard reference materials (river sediment, estuarine sediment, and pine needles). Our mean measured concentration and 95% confidence interval for each NIST reference material were within the certified ranges, with 108 of the 125 individual measurements within the certified ranges. All analyses of NIST river sediment yielded concentrations within the certified range. For estuarine sediments, 15 of 42 analyses yielded concentrations greater than, but within 12% of, the certified range. For pine needles, 2 of 41 analyses yielded concentrations exceeding, but within 5% of, the certified range. The recovery of mercury from analyses of 121 procedural spiked sediment samples averaged 101 ± 2% (95% CI) and ranged from 82 to 128%. Method precision (relative standard deviation), estimated from analyses of 37 sets of triplicate subsamples of sediment from the study lakes, averaged 6% and ranged from <1 to 13%. Mercury concentrations exceeded our calculated method detection limit (American Public Health Association et al. 1989) of 0.7 ng/g (dry weight) in 99% of the 300 sediment samples and exceeded the limit of quantitation (1.8 ng/g) in 95%. For statistical estimation, a concentration value of half the method detection limit was assigned to the three samples (1% of the 300 samples analyzed) with mercury concentrations less than the method detection limit.

Data Analysis

The mean volatile matter and mercury concentrations of surficial sediments in each lake were weighted for the proportion of the lake surface area within each depth stratum (Gilbert 1987). The bulk density of sediment was calculated from the measured water content and volatile matter concentration in the sample, as described in Håkanson and Jansson (1983). The volumetric concentrations (mass per volume of wet sediment) of volatile matter and mercury were calculated from their corresponding dry-weight concentrations and the water content and the calculated bulk density of the sample. The whole-lake burden of mercury in the uppermost 5 cm of sediment in each lake was calculated with the contaminant inventory procedure described by Gilbert (1987).

Statistical analyses were performed with the StatPac Gold® Statistical Analysis Package (Walonick Associates, Inc. 1988). Spearman (rank) correlation coefficients were calculated, by lake, for the following pairs of variables: volatile matter and mercury concentration, water depth and volatile matter concentration, and water depth and mercury concentration. Correlations involving the mercury or volatile matter concentration of sediments were calculated for both dry-weight and volumetric concentrations. Spearman correlation coefficients were also used to examine relationships between areal burdens of mercury as the dependent variable and areal burdens of volatile

TABLE 2. Characteristics of the uppermost 5 cm of sediments in each study lake. The weighted mean, range (in parentheses), and standard deviation of the mean (below the ranges) are given for each variable. Lakes are listed in order of increasing mean pH.

Lake	Water (%)	Bulk density (g/cm^3)	Volatile matter		Mercury	
			% dry wt	mg/cm^3	ng/g dry wt	ng/cm^3
Max	70	1.28	28	32	88	11.6
	(21–98)	(1.01–1.93)	(1–67)	(8–100)	(3–245)	(3.4–36.4)
	3	0.03	1	3	6	0.9
Russett	84	1.11	43	40	157	15.7
	(34–98)	(1.00–1.78)	(2–69)	(17–86)	(5–272)	(4.8–35.6)
	4	0.05	4	2	10	0.7
Little Rock[a]	80	1.16	31	36	97	11.1
	(21–95)	(1.01–1.92)	(1–61)	(14–196)	(6–205)	(4.7–26.1)
	4	0.05	2	4	7	0.6
Crystal	60	1.41	19	17	56	5.2
	(21–97)	(1.01–1.93)	(1–50)	(4–62)	(<0.7[b]–170)	(<MDL–14.6)
	2	0.02	1	1	3	0.4
Vandercook	65	1.34	19	19	63	7.1
	(22–96)	(1.02–1.90)	(1–45)	(1–60)	(2–184)	(0.1–15.3)
	3	0.03	1	1	5	0.4
Pallette	60	1.43	25	17	53	3.3
	(18–97)	(1.01–2.00)	(1–73)	(7–61)	(<0.7[b]–189)	(<MDL–12.1)
	1	0.01	1	1	3	0.2

[a]Reference basin.
[b]Minimum value was less than our method detection limit (MDL) of 0.7 ng/g dry weight.

matter and selected lake characteristics (epilimnetic pH, DOC, conductance, and chlorophyll *a*) as independent variables. A Type I error (α) of 0.05 was used to judge the significance of all statistical tests.

Results

The water content of sediment within each lake ranged from about 20 to 95%, and the mean water content of sediments in individual lakes varied from 60 to 84% (Table 2). The mean bulk density of sediment ranged from 1.11 g/cm^3 in Russett Lake to 1.43 g/cm^3 in Pallette Lake and was inversely related to mean water content. Within each lake, the bulk density of sediments from shallow waters greatly exceeded that of sediments from deeper waters (Fig. 1).

The mean volatile matter concentration (percent of dry weight) in sediments varied considerably among lakes, ranging from 19% in Crystal and Vandercook lakes to 43% in Russett Lake (Table 2). The distribution pattern of volatile matter among the lakes was similar, whether volatile matter was expressed on a volumetric or dry-weight basis.

Mercury concentrations in surficial sediments varied widely within each lake (Table 2). The minimum dry-weight concentrations of total mercury ranged from <0.7 ng/g (our method detection limit) in Crystal and Pallette lakes to 6 ng/g in Little Rock Lake. Maximum dry-weight concentrations ranged from 170 ng/g in Crystal Lake to 272 ng/g in Russett Lake. Mean concentrations of mercury ranged from 53 ng/g dry weight in Pallette Lake to 157 ng/g in Russett Lake. Trends among lakes in mean volumetric concentrations of mercury were similar to those for mean dry-weight concentrations (Table 2). The total mercury concentration of sediment, whether expressed on a dry-weight or volumetric basis, was strongly correlated with the volatile matter concentration of sediment (r_s for the six lakes ranged from 0.50 to 0.93; Table 3).

Concentrations of both mercury and volatile matter, when expressed on a dry-weight basis, were positively correlated with water depth in all lakes (Table 3); that is, dry-weight concentrations increased with increasing water depth (Fig. 2). When expressed on a volumetric basis, however, the concentration of volatile matter was positively correlated with water depth in only two lakes (Crystal and Vandercook), and the concentration

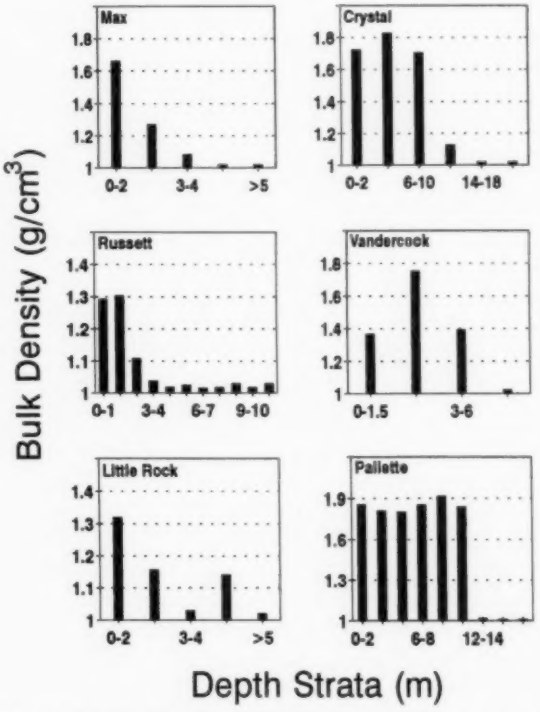

FIG. 1. Bulk density of surficial sediment (uppermost 5 cm) in relation to water depth of each study lake in north-central Wisconsin.

TABLE 3. Spearman correlation coefficients (r_s) between characteristics of surficial sediments, as follows: volatile matter (VM), total mercury (Hg), and depth of water (Z) overlying the sediment. Subscripts denote concentrations based on dry weight (dw) or volume (vol).

	Pair of variables in correlation[a]					
Lake	VM_{dw}:Hg_{dw}	VM_{vol}:Hg_{vol}	Z:VM_{dw}	Z:Hg_{dw}	Z:VM_{vol}	Z:Hg_{vol}
Max	0.85**	0.64**	0.81**	0.72**	−0.04	−0.09
Russett	0.60**	0.73**	0.42**	0.77**	−0.19	0.19
Little Rock[b]	0.87**	0.71**	0.77**	0.78**	−0.09	0.08
Crystal	0.70**	0.87**	0.73**	0.85**	0.58**	0.61**
Vandercook	0.93**	0.92**	0.81**	0.83**	0.55**	0.49**
Pallette	0.64**	0.50**	0.64**	0.83**	0.22	0.61**

[a]Two asterisks indicate significance of r_s at $p < 0.001$; no asterisks indicate nonsignificance ($p > 0.05$); $n = 50$ for each r_s.
[b]Reference basin.

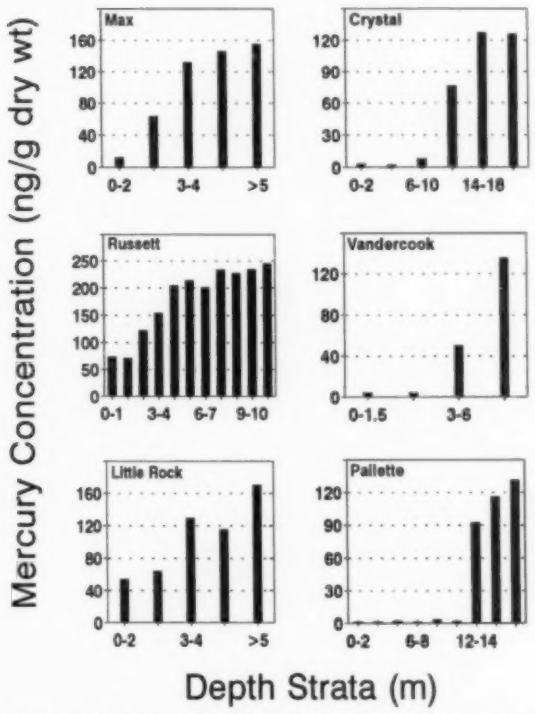

FIG. 2. Depth distribution of mercury (mean values expressed on a dry-weight basis) in surficial sediment (uppermost 5 cm) in the six study lakes in north-central Wisconsin.

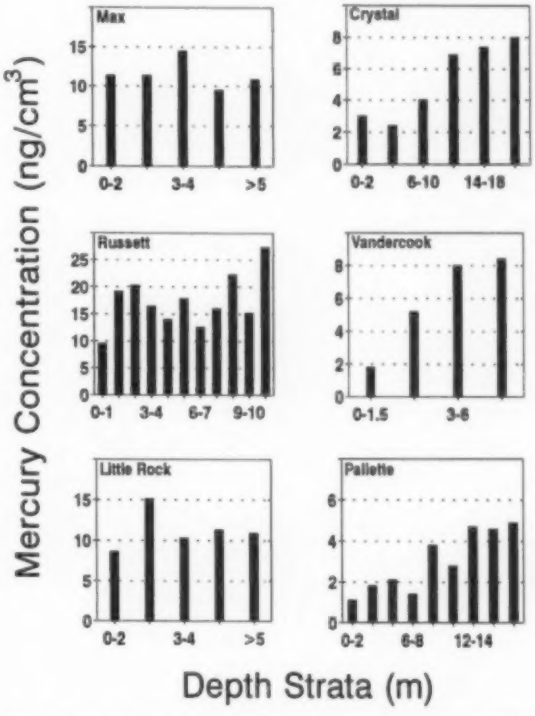

FIG. 3. Depth distribution of mercury (mean values expressed on a volumetric basis) in surficial sediment (uppermost 5 cm) in the six study lakes in north-central Wisconsin.

of mercury was correlated with water depth in three (Crystal, Vandercook, and Pallette lakes; Table 3). Moreover, water depth was less well correlated with the volumetric concentrations than with dry-weight concentrations of volatile matter and mercury in all six lakes.

The depth distributions of mercury in surficial sediments in Max, Russett, and Little Rock lakes, based on volumetric concentrations (Fig. 3), differ markedly from those based on dry-weight concentrations (Fig. 2). The estimated areal burdens of mercury in the uppermost 5 cm of sediment (Fig. 4) paralleled the depth-distribution pattern observed with the volumetric concentrations of mercury (Fig. 3).

Estimated whole-lake burdens of mercury in the uppermost 5 cm of sediment varied from 45 g in Little Rock Lake to 149 g in Russett Lake (Table 4); however, the six lakes ranged nearly ninefold in size (Table 1). Normalized to lake area, the areal burdens of mercury in sediment varied almost fivefold among lakes, from 1.6 g/ha in Pallette Lake to 7.8 g/ha in Russett Lake (Table 4). The areal burden of mercury was strongly correlated with the areal burden of volatile matter in the sediment ($r_s = 0.89$, $p < 0.02$) and with lake pH ($r_s = -0.89$, $p < 0.02$), but was not significantly correlated with epilimnetic DOC ($r_s = 0.20$), conductance ($r_s = -0.37$), or chlorophyll a ($r_s = 0.09$).

The depth distribution of mercury (Fig. 4) and volatile matter (Fig. 5) in the surficial sediments varied considerably among

TABLE 4. Whole-lake and areal burdens of total mercury and volatile matter in the uppermost 5 cm of sediments in the study lakes, listed in order of increasing pH.

Lake	pH	Mercury burden[a]		Volatile matter burden[a]	
		Whole lake (g/lake)	Areal (g/ha)	Whole lake (t/lake)	Areal (t/ha)
Max	5.16	53 (4.0)	5.8	145 (11.9)	15.8
Russett	5.77	149 (7.0)	7.8	379 (18.5)	20.0
Little Rock[b]	6.12	45 (2.5)	5.6	147 (17.9)	18.2
Crystal	6.34	80 (6.7)	2.6	258 (21.0)	8.4
Vandercook	6.37	141 (7.5)	3.5	384 (20.5)	9.6
Pallette	7.03	115 (6.8)	1.6	596 (30.2)	8.5

[a]Standard deviation of the estimated whole-lake burden given in parentheses.
[b]Reference basin.

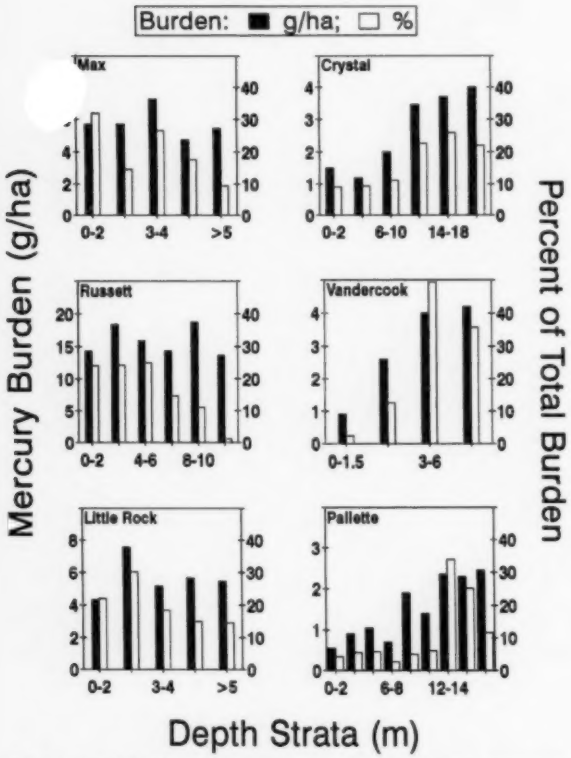

FIG. 4. Areal burdens of mercury in surficial sediment (uppermost 5 cm) in the six study lakes in north-central Wisconsin, in relation to water depth (solid bars are g/ha; open bars are percent of the whole-lake burden present in individual depth strata).

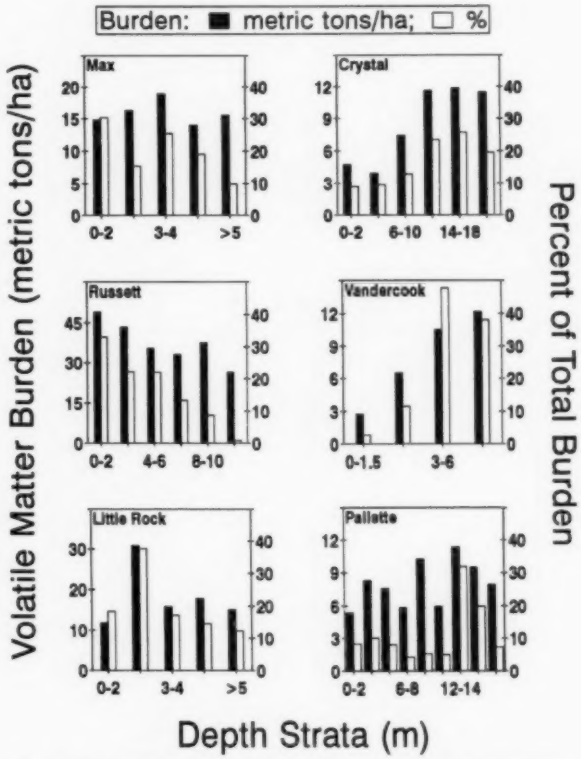

FIG. 5. Areal burdens of volatile matter in surficial sediment (uppermost 5 cm) in the six study lakes in north-central Wisconsin, in relation to water depth (solid bars are t/ha; open bars are percent of the whole-lake burden present in individual depth strata).

the lakes. Areal burdens of mercury increased with water depth in Crystal, Vandercook, and Pallette lakes, but not in Max, Russett, and Little Rock lakes (Fig. 4). The depth distribution of mercury in these lakes closely paralleled the depth distribution of volatile matter (compare Fig. 4 and 5).

Discussion

The sampling design used in the present study yielded precise estimates of whole-lake burdens of mercury, as indicated by the standard deviations of these estimates (Table 4), which ranged from 4.7 to 8.4% of the whole-lake burdens. With a stratified random sampling design, a sample size (N_s) of 50 sediment samples per lake seems ample for estimating sedimentary burdens of mercury in small (≤70 ha) lakes.

Our sampling design, which allocated greater sampling effort to deeper sediments, was based on a priori information on dry-weight concentrations of mercury in surficial sediments (Wiener et al. 1990a). Given the depth distribution of mercury in the surficial sediments of these lakes (Fig. 4), this sampling design was appropriate for the three deeper lakes (Crystal, Pallette, and Vandercook; range of mean depth 4.7–10.4 m). In the three shallower lakes (Little Rock, Max, and Russett; mean depth ≤3.1 m), the areal burdens of mercury in surficial sediments

were nearly uniform among the water-depth strata (Fig. 4); for these lakes, a sampling design allocating equal sampling effort per unit area among strata would have been more appropriate. Even so, our estimates of whole-lake burdens were acceptably precise for all lakes studied, presumably due to the large sample size (N_s). Nonetheless, we advise that decisions regarding allocation of sampling effort be based on volumetric, rather than dry-weight, concentrations of mercury in sediment, when feasible.

Concentrations of mercury in surficial sediments from the profundal zone or underlying the deeper waters ranged from 170 to 272 ng/g dry weight in the study lakes, similar to concentrations reported for other semiremote lakes in north-central Wisconsin (Rada et al. 1989), northeastern Minnesota (Meger 1986; Sorensen et al. 1990), and Ontario (Evans 1986; Johnson 1987). However, the lowest concentrations observed in some shallow-water sediments in our study (<1–6 ng/g dry weight) were less than the minima reported for lakes in Minnesota and Ontario. We attribute the low concentrations in some of our shallow-water sediments, particularly those from Crystal, Vandercook, and Pallette lakes, to the composition of the substrate; these samples consisted mostly of fine gravel, coarse sand, or packed clay, material presumably from the original lake bottom. The strong correlations that we observed between concentrations of mercury and volatile matter in sediments have also been reported by others (Evans 1986; Rada et al. 1986; Wiener et al. 1990a).

The spatial distribution of sedimentary mercury is partly a function of lake morphology, a conclusion supported by our data. The correlations of dry-weight concentrations of volatile matter and mercury with water depth in the six study lakes indicate that fine-grained organic particles accumulate in the deeper zones (Davis and Ford 1982; Häkanson and Jansson 1983; Hilton 1985; Petticrew and Kalff 1991) and that the associated mercury is physically transported to deepwater sediments. Evans (1986) similarly reported that the dry-weight concentration of mercury in surficial sediment of several Ontario lakes increased with increasing water depth. Evans (1986) noted, however, that this correlation with water depth was strong in a deep lake and weak or nonsignificant in shallow lakes.

Dry-weight concentrations (Fig. 2) did not accurately reflect the depth distribution of mercury in surficial sediments of our study lakes (Fig. 4). Thus, consideration of sedimentary mercury content solely on a dry-weight basis could mislead one to underestimate the potential importance of the shallow-water sediments as a source of bioavailable mercury. Similarly, Bird and Duarte (1989) showed that data on microbial activity in sediments were more informative when expressed in volumetric or areal units, rather than per dry weight of sediment.

The bioavailability of mercury in the shallow-water sediments is probably enhanced by the higher temperatures in the epilimnion, which allows greater microbial methylation of mercury. In a comparison study, Winfrey and Rislove (1991) observed significant methylation of ^{203}Hg near the sediment–water interface in the epilimnia of these lakes. In remote lakes in northwestern Ontario, the higher temperatures in the epilimnion (relative to the hypolimnion) stimulated the microbial production of methylmercury in the epilimnetic sediments (Ramlal et al. 1993). On a whole-lake basis, more than 90% of the methylmercury production occurred in the epilimnia of the Ontario lakes (Ramlal et al. 1993).

Three lakes in our study (Max, Vandercook, and the reference basin of Little Rock), whose maximum depths ranged from 6.1 to 7.2 m, did not stratify strongly at any time of the year (Bloom et al. 1991; Webster et al. 1993; P.J. Garrison, Wisconsin Department of Natural Resources, Madison, WI, personal communication). The other three lakes (Russett, Pallette, and Crystal), with maximum depths ranging from 11 to 20 m, underwent pronounced stratification. The depths of the epilimnia were 10 m in Crystal and Pallette lakes and 4 m in Russett lake (J.P. Hurley, Wisconsin Department of Natural Resources, Madison, WI, personal communication). Given the basin morphometries and the mercury distributions in surficial sediments of these lakes, we estimate that the following proportions of the whole-lake burdens of mercury were in the epilimnetic sediments: 48% (72 g of Hg) in Russett Lake, 23% (27 g) in Pallette Lake, and 30% (24 g) in Crystal Lake.

Release of mercury from the sediments could greatly increase the amount of mercury available to biota in these lakes. The whole-lake burdens of mercury in the uppermost 5 cm of sediment, which ranged from 45 to 149 g, greatly exceeded the masses of mercury in other biotic and abiotic compartments in the lakes. In Little Rock Lake, for example, estimated inventories of total mercury in the treatment basin were about 10 g in the uppermost centimetre of surficial sediment, 0.15 g in fish, 0.3 g in water, and 0.1 g in seston (Wiener et al. 1990a; Fitzgerald et al. 1991). The atmospheric input of total mercury to the 10-ha treatment basin was about 1 g/yr, a flux about equal to the net annual sedimentation of mercury (Fitzgerald et al. 1991). The downward flux of atmospherically derived mercury through the water column to the sediment–water interface in Little Rock Lake involved the settling of particles and was greatest during spring and fall plankton blooms (Hurley et al. 1991; Hurley et al. 1993). The transport of ^{210}Pb and ^{210}Po to the sediments of Crystal Lake, studied by Talbot and Andren (1984), was also strongly coupled to the settling of biogenic particles.

Our findings indicate that the retention of mercury is less in high-pH lakes than in low-pH lakes. The areal burdens of mercury in the surficial sediment varied considerably among lakes, from 1.6 to 7.8 g/ha, and were strongly and negatively correlated with lake pH. It is improbable that this fivefold variation in areal burdens of mercury among lakes resulted from differences among lakes in total atmospheric influxes of mercury. The six lakes were in close proximity, and the total atmospheric influxes of mercury, per unit of surface area, were probably similar among lakes. Although differences in sedimentation rates among the lakes may explain some of the fivefold variation in areal burdens of mercury in surficial sediments, we lack the information needed to assess this directly.

We hypothesize that the pH-related variation in areal burdens of mercury was partly caused by differences among lakes in the pH-related efflux of gaseous mercury (Hg^0) from the water column to the atmosphere (Vandal et al. 1991). The volatilization of mercury, almost entirely as Hg^0, removed geochemically significant amounts of both the reactive mercury and the total mercury entering these lakes in atmospheric deposition (Fitzgerald et al. 1991; Vandal et al. 1991). Xiao et al. (1991) similarly estimated that a significant part of the total atmospheric influx of mercury to lakes in southern Sweden may be released from the lakes through volatilization.

Surface waters of our study lakes were strongly supersaturated with Hg^0 (Fitzgerald et al. 1991; Vandal et al. 1991). The mechanisms that produce Hg^0 have not been defined, but seem to be enhanced by high pH and high dissolved oxygen concen-

tration (Vandal et al. 1991). Thus, the flux of Hg^0 from the water column to the atmosphere would seem to correlate positively with pH.

One of us (D.E. Powell, Iowa State University, Ames, IA, unpublished data) has analyzed surficial sediments (uppermost 5 cm) from these lakes for cadmium. The areal burdens of cadmium ranged from 62 to 92 g/ha in the six lakes. The 1.5-fold variation in areal burdens of cadmium among lakes was small relative to the fivefold variation reported here for mercury. In contrast with the situation for mercury, it is improbable that significant cadmium is lost through volatilization from the water column to the atmosphere. If there were no efflux of volatile Hg^0, we would expect areal burdens of mercury in the surficial sediments to vary little among lakes. Moreover, the similarity of the areal burdens of cadmium among lakes suggests that sedimentation rates do not vary greatly among the six lakes.

Two studies of the stratigraphy of mercury in lacustrine sediments (Evans 1986; Johnson 1987) provide further indirect evidence that less mercury is deposited to the sediments in alkaline lakes than in low-pH or circumneutral lakes. Johnson (1987), who analyzed cores from 14 Ontario lakes, found that surficial sediments in three lakes, all having high ANC (pH range 7.5–8.8; ANC 1360–3080 μeq/L), did not exhibit the mercury enrichment typically observed in North American lakes (Norton et al. 1990). A similar situation was observed by Evans (1986), who analyzed sediment cores from 14 remote lakes in south-central Ontario. Surficial sediments were enriched with mercury in all lakes but one, a marl lake with sediments rich in $CaCO_3$.

Several surveys have revealed considerable pH-related variation among lakes in the mercury concentration in resident fish (Spry and Wiener 1991). In three such surveys of Wisconsin seepage lakes, low-pH and high-pH lakes were interspersed and in close proximity, and variation in the mercury content of fish could not logically be attributed to differences in the atmospheric deposition of mercury among lakes (Cope et al. 1990; Wiener et al. 1990b). Variation in the bioaccumulation of mercury among freshwaters has been partly attributed to pH-related variation in the microbial production of methylmercury (Xun et al. 1987; Winfrey and Rudd 1990; Miskimmin et al. 1992). The formation of Hg^0, leading to the efflux of mercury from lakes to the atmosphere, may be another internal process that significantly affects the quantity of mercury available for methylation and for subsequent uptake by freshwater biota.

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PERSPECTIVES

Current Trends in Including Risk and Uncertainty in Stock Assessment and Harvest Decisions[1]

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Hilborn, R., E.K. Pikitch, and R.C. Francis. 1993. Current trends in including risk and uncertainty in stock assessment and harvest decisions. Can. J. Fish. Aquat. Sci. 50: 874–880.

Many fisheries management bodies are considering methods for explicit consideration of uncertainty and risk in harvest decisions. We propose a two-step process where, in the first step, a stock assessment group determines the possible biological and economic "states" of the fishery and evaluates the expected outcomes of different possible management actions for each possible "state." The evaluation may be summarized in a tabular form, or it can take the form of a computer model. In the second step, a decision-making body, consisting of government, public, and user-group representatives, examines the risks and benefits of each possible management action, given the possible "states" of the fishery, and formulates a decision. This proposal differs from many current stock assessment and management procedures in that (1) the stock assessment group makes no recommendations about catch levels, (2) the stock assessment group does not attempt to produce a "best" estimate of stock condition, and (3) the decision group deals explicitly with risk and biological and economic uncertainties.

De nombreux organismes de gestion des pêches envisagent l'utilisation de méthodes permettant de tenir compte explicitement de l'incertitude et du risque dans les décisions relatives à la récolte. Nous proposons une méthode en deux étapes. Au cours de la première étape, un groupe d'évaluation des stocks détermine les « états » biologiques et économiques possibles de la pêcherie et évalue les résultats attendus de différentes mesures de gestion possibles pour chacun des « états » possibles. L'évaluation peut être résumée sous forme de tableau ou sous forme de modèle informatique. Dans la seconde étape, un organisme de prise de décision, formé de représentants du gouvernement, du public et des groupes d'usagers, examine les risques et les avantages de chacune des mesures de gestion possibles, compte tenu des « états » possibles de la pêcherie, et formule une décision. Cette proposition diffère des nombreuses méthodes courantes d'évaluation et de gestion des stocks du fait que 1) le groupe d'évaluation des stocks ne formule aucune recommandation sur les prises, 2) le groupe d'évaluation des stocks n'essaie pas de produire la « meilleure » estimation de l'état du stock et 3) le groupe qui prend la décision traite exclusivement des risques et des incertitudes biologiques et économiques.

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Fisheries management is primarily a decision process. Managers make a choice between alternative actions in an effort to reach desired social, economic, and biological objectives. A key element of fisheries stock assessment is the uncertainty associated with estimates of current stock size and potential productivity. The uncertainty in assessment leads to risk in decision making. If quotas are set high and the stock is low, managers risk driving the stock down and the need for future reductions in catch. If quotas are reduced, and the stock is actually large, managers risk economic damage to the fishing industry. The purpose of this paper is to show how stock assessment and decision making can be coordinated to deal with the risk and uncertainty inherent in fisheries management. We first briefly summarize the process by which fisheries management decisions are commonly derived and present our view of some of the failings of current practice. We then illustrate the potential consequences of these failings with a discussion of the northern cod controversy. Finally, we present a proposal for an alternative decision-making process in which the decision group, in concert with the assessment group, explicitly deals with uncertainty and risk.

The need for explicit recognition and analysis of uncertainty and risk is now widely acknowledged (McGlade and Shepherd 1992; Pearse and Walters 1992) and Atlantic fisheries agencies such as the Canadian Atlantic Fisheries Scientific Advisory Committee (CAFSAC) and the North Atlantic Fisheries Organization (NAFO) have held a number of meetings in recent years to consider these topics. Regier and McCracken (1975) recognized the need to separate the assessment process from the politics of decision making and recommended structures that were similar to those that have been adopted, although their proposals did not discuss mechanisms for dealing with risk and uncertainty. We have been working with the New Zealand Fishing Industry Board (NZFIB) and the New Zealand Ministry of Agriculture and Fisheries (NZMAF) to implement many of the proposals contained in this paper.

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Stock Assessment, Biologists, and Managers — The Current Situation

Many fisheries management agencies have adopted rather similar structures to use the products of stock assessment. A "scientific committee" reviews the available biological data, analyzes the consequences of proposed alternative fisheries actions, and makes a recommendation about the next year's quota, season length, or other regulations that are employed in the fishery. The scientific committee then passes its current best estimate of the state of the stock and a recommended regulation to a "decision group" whose decision may differ from that of the scientific committee because of political, social, or economic considerations.

Figure 1 shows the decision-making steps for groundfish management employed by the U.S. Pacific Fisheries Management Council (PFMC) which manages fish stocks off the coasts of California, Oregon, and Washington. First, a groundfish management team, composed of biologists and economists from state and federal agencies, does the technical stock assessment and then recommends an allowable biological catch (ABC) based on their analysis. This ABC is normally a single number, although on occasion a range may be given. The ABC and supporting analyses are then passed on to the Scientific and Statistical Committee (SSC) which includes biologists, economists, and other social scientists and may recommend an ABC that differs from that proposed by the team and then passes this recommendation on to the actual Council. The Council is composed of senior government administators and at-large members appointed by the governors of each state who make a final decision on the quota. The process employed by the PFMC is typical of many fisheries decision processes around the world.

To illustrate the widespread use of these processes, Table 1 summarizes the membership and products of a number of fisheries decision-making bodies. In the eastern Atlantic prior to 1980, the International Council for the Exploration of the Sea (ICES) did stock assessment and the Northeast Atlantic Fisheries Commission (NEAFC) managed several stocks with an assessment and decision-making process quite similar to those in Table 1 (Saetersdale 1980).

Problems with Current Practice

We see a number of major problems with the existing structure.

(1) Decision makers rarely deal explicitly with uncertainty. There is almost always considerable uncertainty about the current stock size and its potential response to alternative catch levels. Retrospective analysis has shown that stock abundance estimates have been off by as much as 100%, as in the case of the northern cod discussed below and for several herring stocks in eastern Canada (Sinclair et al. 1985). In our experience, uncertainties about current stock size are rarely fully and explicitly transmitted to, or considered by, the decision group. Instead, the main product of the stock assessment group is often its best estimate of stock abundance.

(2) The decision group has difficulty evaluating new options. At best, the stock assessment group might provide a table of options and anticipated outcomes. Rarely, if ever, do the nature and time constraints of the process allow a decision group to determine the likely outcome of an alternative option not considered by the assessment group. Evaluating new options requires some form of computer gaming, and such gaming is common practice among stock assessment biologists (McGlade and Shepherd 1992), at least when they are individually preparing for meetings. Unfortunately, decision makers do not usually have this opportunity, and computer gaming is not part of the institutional process of policy evaluation. Yet it is precisely the decision makers who should have the opportunity; it is they who should examine dozens of alternative policies under different biological hypotheses.

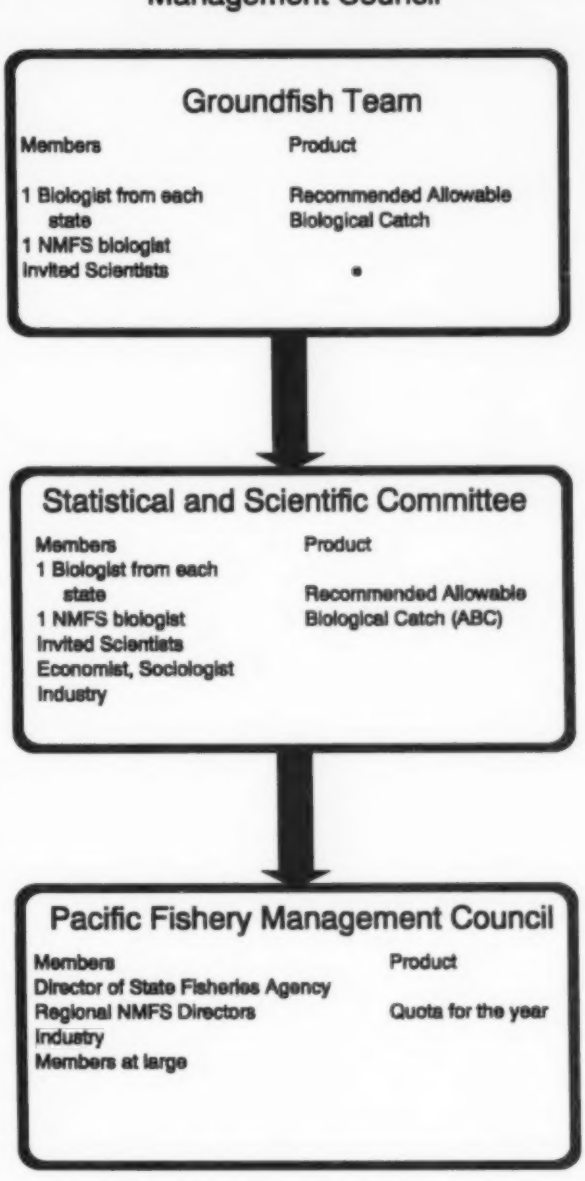

FIG. 1. Steps in the decision process in the PFMC for groundfish. The PFMC manages the ocean fisheries of California, Oregon, and Washington. NMFS stands for National Marine Fisheries Service, the U.S. federal fisheries agency.

TABLE 1. Fisheries organizations and the composition and products of their scientific and decision groups. IPHC = International Pacific Halibut Commission, IWC = International Whaling Commission, ICES = International Council for the Exploration of the Sea, CEC = Council of the European Community, PSARC = Pacific Stock Assessment Review Committee, CAFSAC = Canadian Atlantic Fisheries Scientific Advisory Committee, ICSEAF = International Commission for Southeast Atlantic Fisheries.

Agency name	Composition of stock assessment group	Product of stock assessment group	Composition of decision group	Product of decision group	Harvest strategy chosen
U.S. councils	Scientists from state and federal agencies	Recommended quota	Directors of state and federal agencies and at-large members usually representing user groups	Annual TAC	Varies
U.S. and Canada halibut IPHC	Scientific staff of commission	Recommended quota	Three commissioners from each country	Quota for each country	20% harvest rate
Southeast Atlantic ICSEAF	Scientists from each country	Recommended quota	Representatives from each country	Quota for each country	Constant harvest rate
Europe ICES and CEC	Scientists from each country	Usually recommended policy	Representatives from each country	Quota for each country	Varies
New Zealand	Government and industry scientists, conservation groups	Extensive evaluation of alternatives	Government and industry decision makers	Annual quota	None adopted
IWC	National and invited scientists	Expected performance of alternative strategies	Representatives from each country	A harvest strategy	Complex
Canada west coast PSARC	DFO scientists, provincial scientists, some user groups	Assessment documents and summaries	DFO managers	Annual quota or fishing regulations	Generally not adopted
Canada east coast CAFSAC	DFO scientists	Assessment documents and summaries	DFO managers who consult with industry before final decision adopted	Annual quotas	Primarily harvest rate policies with smoothing mechanisms

(3) Many policy options may be eliminated before beginning the decision phase. Different harvest strategies will produce differences in average yield, variability of yield, average catch-per-unit-effort (CPUE), average stock size, etc. Who should determine the extent to which higher average yields are worth the attendant higher yield variability? In practice, this choice may be taken at the stock assessment rather than the decision level. Choice of harvest strategy is a major policy decision and thus should more appropriately be assigned to the decision makers.

(4) The composition of the stock assessment and decision groups may provide little representation for user groups. In some agencies, there is absolutely no user-group representation; in others, there is some in the decision phase. Yet many of the choices involved — particularly between average yield, variability of yield, and average CPUE — most directly affect the users.

(5) If a harvest strategy is in place, a revision in the stock abundance or productivity estimate may cause the estimated catch quota for that year to be beyond the range of values considered when the strategy was adopted. Many harvest strategies (including the Canadian Atlantic Fisheries Groundfish Management Plan discussed below) include rules to buffer dramatic changes in quota as stock assesments are revised.

The Northern Cod Controversy

The northern cod is the major fishery in eastern Canada and is found off Labrador and eastern Newfoundland. The stock has been fished since 1481 (Harris 1990) and has been the mainstay of much of Newfoundland, both for small-boat inshore fishermen and, more recently, large offshore trawlers. Intense fishing by foreign fleets in the 1960s produced the highest recorded catch of about 800 000 metric tons (t) in 1968, which soon led to a dramatic decline in the stock and a collapse of the inshore catch.

In 1977, Canada extended jurisdiction to 200 miles and thereby almost all of the northern cod fell under Canadian juris-

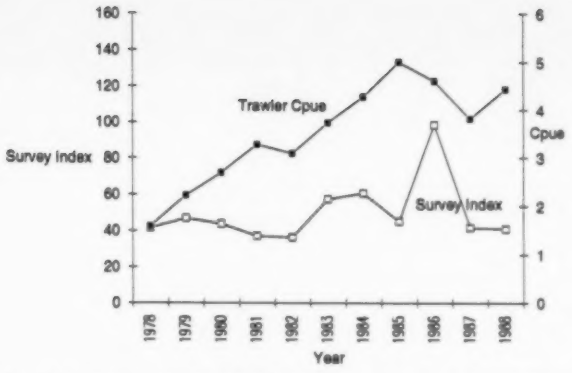

FIG. 2. Trends in trawler CPUE and survey indices for the northern cod from 1978 to 1988. Data from CAFSAC 1989.

diction. Canada then took over the existing International Commission for Northwest Atlantic Fisheries (ICNAF) program of stock assessment and monitoring and instituted an $F_{0.1}$ harvest strategy which was intended to result in a catch of roughly 20% of the exploitable biomass every year. The major data for stock assessment are catch-at-age data, CPUE of Canadian trawlers, and research surveys. Although both the CPUE and survey data showed that the stock was increasing from the low in the early 1970s, the two measures indicated different stock sizes (CAFSAC 1989). The CPUE indicated a substantially larger stock recovery than the surveys (Fig. 2).

This discrepancy greatly concerned the stock assessment biologists; however, the 1986 surveys indicated a much larger stock than previously estimated (CAFSAC 1989) and they seemed to be in agreement with the CPUE. In 1987, the quota was raised to 266 000 t (from 240 000 t), which was thought to be below the $F_{0.1}$ quota of 290 000 t. The quota was set lower than 290 000 t because of concerns about the reliability of the data and other factors. For instance, while the total catch of northern cod had been increasing, the inshore fishermen had encountered very low CPUE, had been unable to catch their allowances for several years, and had been pressing for lower offshore trawl total allowable catches (TACs). The choice of 266 000 t (rather than 290 000 t) was probably due in part to pressure to reduce offshore quotas.

Department of Fisheries and Oceans (DFO) scientists were faced with differing signals: trawler CPUE was increasing, surveys were relatively constant, except for the 1986 survey, and inshore CPUE was decreasing. Up to 1987 the stock assessment method used had been to choose the average abundance estimate of the research survey and trawler CPUE (CAFSAC 1989). This averaging was the outcome of long and extensive analysis and discussion of the relative reliability of the two indices and, by all external reviews (Harris 1990), reflects the best available stock assessment methods. Once the weightings for the different indices of stock abundance were chosen, the allowable catch could be calculated using the $F_{0.1}$ harvest rate, but, as mentioned above, the quota was set slightly lower than indicated by $F_{0.1}$.

Unfortunately the 1987 and 1988 research surveys again indicated lower stock sizes (roughly half of what was estimated in 1986), and the CPUE data for 1987 and 1988 were down. In 1989, CAFSAC announced that it had revised the estimates of stock size downward and under the $F_{0.1}$ strategy, this would result in a major drop in TAC.

The ramifications of this revision in stock assessment were dramatic. Some senior officials within DFO lost their jobs, the Minister moved on to another portfolio, a panel of inquiry was appointed to investigate (Harris 1990), and senior politicians pontificated on the radio that they would never trust fisheries scientists again. But most important, the social and economic lives of Eastern Canadian fishermen were on the line: if TACs were actually cut, thousands of jobs would be lost and numerous enterprises would go bankrupt.

The case of the northern cod in the 1980s is clearly a failure of fisheries management. This is the most important fishery in Canada, and Canada is one of the most advanced countries in the world in fisheries stock assessment. What went wrong?

In his review, Harris (1990) took direct aim at the stock assessment scientists: "In those circumstances, scientists, lulled by false data signals and, to some extent, overconfident of the validity of their predictions, failed to recognize the statistical inadequacies of their bulk biomass model and failed to properly acknowledge and recognize the high risk involved with state-of-the-stock advice based on relatively short and unreliable data series." However, we think that there was no real failure in stock assessment. The biologists knew that there were (at least) two alternative views of the stock trend and they had repeatedly stated this in their stock assessment documents. In retrospect, there was no major indication of a real crisis with northern cod in 1988. The revised assessments said that the stock had been exploited at roughly 40% (instead of the previously estimated 20%), but nevertheless, stock abundance appeared to be increasing or stable. Thus, there was no stock collapse, and since the stock appeared to be stable or increasing at a 40% harvest rate, there did not appear to be any pressing need to reduce the quotas dramatically.

In our view, Harris's quote does highlight two critical aspects of the stock assessment process, the *uncertainty* of stock assessment ("statistical inadequacies") and the *risk* in decision making. Eliminating uncertainty and risk cannot be done, but both can be dealt with successfully in the assessment and decision-making process.

Simply stated, CAFSAC (and earlier, ICNAF) followed the averaging route and never explicitly dealt with risk. We do not think that the stock assessments could have been done better, but we do think that the stock assessment and management system was poorly designed and we believe that there is a better way.

Embracing Risk and Uncertainty

The most important step in redesigning a stock assessment and management system is to recognize that arriving at a management decision (TAC in the case of northern cod) is a process that involves taking risk in the face of biological, economic, and social uncertainty. Uncertainty and risk must not be swept under the carpet but must be explicitly dealt with. The simplest format for doing this is a decision table (Walters and Hilborn 1976; Walters 1986; Hilborn and Walters 1992). Table 2 shows a sample decision table from our work on the hoki (*Macruronus novaezelandiae*) fishery in New Zealand. The biological/economic/social alternative system "states" are laid out as columns, and the possible decisions are rows. In Table 2 the possible states are estimated virgin stock sizes, and several possible harvest actions are evaluated as rows. The entries in the cells of a decision table are the expected consequences of the action if the "state" happens to be true. These consequences

TABLE 2. Sample decision table showing the expected average yield over a 5-yr time horizon (in thousands of metric tons) for three possible states of nature and four possible actions. The possible states of nature are virgin biomasses of 0.9, 1.5, and 2.1 Mt with probabilities of 0.57, 0.40, and 0.03, respectively. The possible actions include constant quotas or specific quotas in 1993 followed by harvest rates.

Policy	Virgin biomass (probability)			Expected value
	0.9 Mt (0.57)	1.5 Mt (0.40)	2.1 Mt (0.03)	
150-kt constant quota	136	150	150	148
150-kt quota in 1993; 39% harvest rate after	137	186	202	168
200-kt quota in 1993; 39% harvest rate after	138	194	216	173
200-kt quota in 1993; 26% harvest rate after	122	154	166	141

may be compressed into a few numbers such as average catch, profits, and jobs or may be graphic projections of different indicators over time. While the word "risk" has come to have a well-defined meaning in some circumstances (Francis 1992), we prefer to think of risk as potentially being both low stock sizes (risk to the stock) and potential decreases in catch or profitability (risk to the fishery).

This decision table should be the centerpiece of the decision-making process: uncertainties and risks are clearly defined and decision makers can collectively weight the risks. While formal decision tools, such as maximization of expected values, can be used in theory, we believe that explicitly looking at the uncertainties and risks is the critical step.

As central as it may be, however, the decision table is only one step in our proposed stock assessment/management process. Someone, after all, has to make up the table. The obvious people to identify alternative "states" are the stock assessment biologists and economists. It is their job to identify the biological and economic possibilities. The stock assessment staff is also generally well qualified to identify at least some of the possible management decisions and to evaluate their consequences.

In a simple world, we can envision a two-step process in which the stock assessment staff identifies possible states of the stock and their relative probabilities, evaluates alternative management actions, and prepares a decision table. This table would thus be the product of stock assessment and it would be passed to the decision makers. The decision makers would weight the probabilities of the different states and the risks associated with different decisions and then decide.

The world is rarely this simple. We do not believe that stock assessment staff would ever be able to anticipate and evaluate the consequences of every possible management alternative, and we know from experience that managers will always ask one more "what if" question. Thus the stock assessment staff cannot simply produce a document and retreat from the scene — the end product of stock assessment cannot be a single decision table. Rather, we believe that the stock assessment process should produce a set of alternative computer models (or alternative parameters of the same model), each one capturing different possible "states." The model would produce the standard biological outputs such as catch, effort, and CPUE, as well as economic indicators of performance such as profitability and employment. A "handler" will present the models to the decision makers who can then try different management actions on the various possible "states." In some cases the decision alternatives may be simple enough that a decision table would suffice — but we suspect that as a rule, decision makers will continue to ask yet another "what if" question. Ideally, there ought to be an iterative series of meetings of the assessment group followed by one for the decision group, but this obviously requires a great deal of time.

The microcomputer revolution is making this interactive gaming approach possible. Powerful laptop computers are now used by many stock assessment biologists, and the types of interactive policy evaluation described above are within the technical grasp of most management groups.

Discussion

Fisheries biologists are understandably reluctant to present alternative possible states because they usually find that decision makers choose the most optimistic scenario as the most likely (Wright 1981). Our own experience has been that, when presented with different possible catch levels, many fishers will tend to choose the higher ones, although this may well be because the implications of this choice are not fully conveyed or understood. However, under our proposal, the stock assessment group does not present alternative catch levels; rather, they present alternative states of the stock and the consequences of alternative catch levels if the different states happen to be the real state. Thus, while higher catch levels would be possible if the most optimistic state were true, they would spell disaster for the most pessimistic one. We believe that decision makers of both government and user groups must examine the risks first and then decide.

We suggest that the decision-making group consist of government officials, user-group representatives, and other public groups with some stake in the fishery. Participation of user groups is common among the organizations listed in Table 1, and one could argue that user groups should be the primary decision makers because they must suffer the consequences of

the decisions. But, we do not think that user groups should be the sole decision makers, primarily because the government and the taxpayers it represents also bare considerable risk. In most countries, the taxpayer pays when a fishery collapses. Governmental risks may be very different from users' risks — when fisheries are highly profitable, the fishers get most of the reward; when fishing is unprofitable, governmental subsidies often keep fishers afloat.

In many fisheries, there are multiple user groups who have different objectives when it comes to allocation, timing of fisheries, gear, etc. Indeed, in many fishery decision groups, recreational users, aboriginals, and environmental groups often have or seek representation. There is no single user voice and the government will have to play the moderating role.

Our proposal differs from the type of decision process commonly used in three significant ways. First, the stock assessment group makes no recommendations about management actions. Rather it explicitly describes the range of biological and economic possibilities that are consistent with the data and evaluates the consequences of actions managers would like to consider. Hilborn and Walters (1992) described methods that can be used to compress the uncertainty into a small number of discrete alternatives, but stock assessment groups will need to find ways both to capture the reality of uncertainty in stock size and productivity and to compress this to manageable proportions that the decision group can understand.

Second, the stock assessment group does not attempt to make any "best" estimates of biological parameters such as maximum sustainable yield (MSY), current stock size, etc. A current trend in stock assessment methods is to attempt to take different, and often contradictory, types of data and "synthesize" these data into a single "best" estimate (Fournier and Archibald 1982; Deriso et al. 1985; Methot 1989; Hilborn and Walters 1992). This can be dangerous if the data are contradictory because the uncertainty inherent in divergent data will tend to be masked by the process of obtaining a single estimate (Richards 1991). In the case of the northern cod, the contradictory signals of CPUE and surveys were averaged: it would have been better if the two alternative hypotheses about stock size had been kept as distinct possibilities and passed on as such to the decision makers. Yet when different data sources are complementary, rather than contradictory, the "synthetic" methods may be very useful. For instance, Hilborn and Walters (1992) showed how the knowledge that a stock was unexploited at the beginning of a data series may dramatically reduce the uncertainty in fitting Schaefer population models to index of abundance data.

Third, our proposal differs from much existing stock assessment practice in that the decision makers have explicit biological/economic alternatives in front of them and the numerical tools (computer simulations) for real-time evaluation of those alternatives. Many stock assessment groups are either now doing this or moving rapidly in this direction.

Will it work? Can decision makers make good use of simulation models and consider a wide number of alternatives? Certainly there are no technical problems: almost all fisheries agencies have staff capable of building simulation models with which the consequences of alternative harvest strategies can be examined. We are currently implementing these methods for a number of fisheries in New Zealand. Thus far, we have used decision tables as the centerpiece for discussion (Cordue et al. 1992) and have begun to use simulation models during working group sessions with decision makers and user groups. On the nontechnical side, there are obstacles to overcome, however. One road to success is the ability of the model builders to work with and communicate with the decision makers. Hilborn et al. (1984) described how it can be done in the process of formulating regulations for a commercial troll and sport fishery in British Columbia. We have been involved in a number of other projects (Hilborn and Luedke 1987, for instance) where models were given to decision makers to analyze and evaluate alternative policies. And our experience has been not only that decision makers can use such tools, they want them!

A second potential problem is the diverse makeup of most fisheries decision-making bodies. Some decision makers may need education and training in the concepts of decision analysis for this process to work. On the other hand, industry members of the group may have considerable experience incorporating risk into decision making, as they frequently deal with market and other "risks" in making everyday business decisions.

We do not underestimate the demands such a system will place on the stock assessment staff. It is blissfully simple to do the analysis, make a recommendation about catch, pass this along to decision makers, and hope for the best whereas the model "handlers" would have to spend much time and expend considerable effort explaining the biological alternatives, developing graphical output for their models, and talking to the decision makers.

Every fishery management system is different, and we have undoubtedly failed to identify numerous problems that will arise. However, the essence of our suggestion, that the stock assessment group transmit the alternative hypotheses to a decision-making group, can be carried out in most circumstances.

Possibly the biggest mental hurdle to overcome is the realization that our new decision process rests on trust and responsibility. Stock assessment biologists must trust the decision makers to make informed decisions, and the decision makers must trust the biological staff to provide them with a realistic appraisal of potential outcomes of different options. The biological staff must be responsible for a frank and realistic assessment of stock status and potential response to alternative actions, and the decision makers must accept the responsibility for making decisions in a difficult and uncertain environment.

Acknowledgments

The ideas described in this paper have been developed while working with the New Zealand Fishery Industry Board (FIB) in examining the fisheries management process in New Zealand. The support of the FIB, and Ray Dobson and Andrew Branson in particular, is gratefully acknowledged. Dr. William Aron provided considerable useful comment on a draft of this paper. Dr. Jake Rice and two anonymous reviewers provided valuable insight on the history of the northern cod fishery, but we take all responsibility for the perspective provided in this paper.

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LETTERS AND COMMENTS / LETTRES ET COMMENTAIRES

Comment on R.I.C.C. Francis: Computing Probability Distributions for Risk Analysis

Francis (1992) provides an elegant methodology for assessing risks of stock collapse in developing fisheries, emphasizing the need to provide probabilistic advice rather than point estimates or best guesses. My concern is with only one technical detail of the methodology, namely a confusion about definitions of "probability" that leads him to recommend a computational procedure that requires hundreds of times more calculation than necessary and contains a hidden assumption about the prior probabilities placed by the analyst on alternative possible values of the initial stock size.

Francis notes that a key step in the analysis is to assign a probability or measure of confidence to eachpossible value for the parameter "initial stock size," given the data. Statisticians approach this problem in two ways. Classical or "frequentist" statisticians argue that it is nonsense to assign a probability to a parameter value in the first place; they contend that probabilities refer to sample spaces, and a parameter is simply a number that is not sampled in the first place. As we are all taught in basic statistics, they restrict their probabilistic statements about parameters to confidence statements of the form "the probability of obtaining the observed parameter estimate is less than X if the true parameter value is in fact more extreme than Y and Z." Bayesian statisticians, on the other hand, define probability more broadly, as "degree of belief" or credibility. Under this broader definition, they are quite happy to assign a probability to a parameter value, but they are careful to point out that this probability logically has to contain two components: (1) a sample-space probability statement about the probability of the data given the parameter value, which is called the likelihood of the data, and (2) a prior or subjective probability of the parameter value, independent of the data. The second component comes from the logic of moving from a conditional statement about the probability of the data given a parameter to a second conditional statement about the probability of the parameter value given the data; basic probability definitions require that this move include a calculation of the total probability of the data, which requires that a prior probability be assigned to each possible parameter value that could have given rise to the data in the first place. The debate between frequentist and Bayesian statisticians is not about this point, but rather about the danger of scientists injecting personal beliefs into analysis through their coice of a prior probability distribution. For further discussions of Bayesian and frequentist approaches, see Jeffreys (1961), Box and Tiao (1973), Good (1983), Berger (1985), and Berger and Berry (1988).

Francis notes that frequentist statements about confidence can be generalized from presenting simplistic confidence limits to presenting a "confidence distribution" displaying the conditional probability of obtaining parameter estimates less than or equal to X for a range of values for X. He outlines a computationally intensive Monte Carlo procedure for generating this distribution. However, he then talks about how this distribution represents "our confidence that the true value" of the parameter lies between any two values, and he goes on to plot and use the confidence distribution with probability labels (Francis 1992, fig. 1) just as though it were the Bayesian posterior distribution (conditional probability distribution of the parameter, given the data). In dropping the conditional terms that should be used when referring to the confidence distribution, he implicitly treats it in further analysis as a Bayes posterior distribution, without telling us about the prior distribution that he had to assume in making the transition. He slips from a frequentist to a Bayesian interpretation of "probability" in order to proceed with the risk analysis. He has in fact assumed an uninformative or uniform priordistribution in making this transition.

The point here is not that Francis is acting like a closet Bayesian; it is laudable that he seeks to make only objective probability statements that are not contaminated by an analyst's subjective prior distribution. The point is that logically he has not escaped the prior distribution, and by recognising this we can devise a computational procedure for the posterior probability distribution that is both orders of magnitude more efficient than his and permits (indeed, forces) explicit statements about the prior odds that we would assign to alternative values of the initial stock size.

The improvement in computational efficiency comes because the Bayes posterior distribution is computed directly from the likelihood function for the data, without the intermediate step of computing the maximum likelihood estimator and estimating its conditional distribution by Monte Carlo procedures. For an uninformative or uniform prior (as Francis has implicitly assumed), the posterior probability for each parameter value is just the likelihood of the data given that value, divided by the sum over the range of all parameters values considered of the likelihoods of the data given each value. For each parameter value in this range, Francis would instead have us do hundreds of simulations, with each involving computation of log likelihoods in order to find a maximum likelihood estimate. This is simply an enormous waste of time to produce a result that is far more difficult to check, and likely no more credible to decision makers who are expected to use it, than the usual Bayes posterior distribution.

There is a general point to be made here. Fisheries assessment calculations are already complex and difficult, and fraught with risks of misunderstanding. As we begin to expand these methods to make use of modern ideas from statistical decision theory and decision making under uncertainty, the last thing we need is a reversion to old methods and debates about frequentist versus Bayesian statistics — Carl Walters, *Fisheries Centre, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.* (JB642)

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The Interpretation of "Probability": A Response to Walters' Comments

I am grateful to Walters (1993) for drawing attention to a portion of my risk analysis paper (Francis 1992) in which I have not been as explicit as I should have been about my assumptions. However, I disagree with some aspects of his interpretation of the analysis. In this note, I will respond to three of Walters' assertions and attempt to clarify my interpretation of "probability".

The first assertion is that I have used "a computational procedure that requires hundreds of times more calculation than necessary". My view is that we should use what appears to be the best procedure for any given problem. It is only when there is little or nothing to choose between two procedures that we should be swayed by the matter of computational intensity (as long, of course, as the amount of computation is not prohibitive). As Efron (1979) pointed out, the availability of high-speed computers liberates us to "think the unthinkable", i.e., to use the power of computers to avoid computational compromises and questionable approximations. To put the computational effort in perspective, I would point out that the orange roughy (*Hoplostethus atlanticus*) fishery of my example generates more than a hundred million dollars annually, and the New Zealand government spends almost a million dollars each year in carrying out the biomass survey that is the major input to the analysis. In this context, I do not think that the few minutes of computer time that are required by the confidence distribution procedure are disproportionate.

Second, Walters states (with no supporting argument) that I tacitly assume an "uninformative or uniform [Bayesian] prior". I do not believe this is true (but would welcome a proof to the contrary). It *is* true that, in some simple cases, the confidence distribution is identical to the Bayesian posterior calculated assuming an uninformative prior (a simple example is the estimation of the mean of a normal distribution of known variance). However, this is not generally true (Kendall and Stuart 1979). In an application of the risk analysis technique to hoki (*Macruronus novaezelandiae*), it was found that the Bayesian posterior was strongly dependent on which uninformative prior was used and that the confidence distribution differed from the Bayesian posteriors for all the priors considered (Cordue et al. 1992).

Incidentally, the Bayesian approach for the example in Francis (1992) is not as straightforward as Walters appears to believe because the prior must be specified for two variables: the virgin biomass, B_0, and the catchability, q. Walters seems to have overlooked the latter parameter (see the end of the last sentence in his first paragraph). The existence of this "nuisance parameter" has two effects. First, it increases the choice of possible uninformative priors (and I agree with Kendall and Stuart (1979, section 21.33) in finding Jeffreys' rules for forming uninformative priors arbitrary). Second, to obtain a posterior distribution for B_0, we must calculate the marginal distribution of the joint posterior in B_0 and q. Usually there will be no way to do this analytically, so numerical integration (computationally intensive!) will be required. It happens, however, that, for this specific problem, a simplifying approximation is available (see appendix in Cordue and Francis 1992).

Third, Walters states that I "slip from a frequentist to a Bayesian interpretation of 'probability' ". It is certainly true that to refer to the curve in fig. 1B of Francis (1992) as a probability distribution for B_0 is not correct according to frequentist theory. However, to call this usage Bayesian is going too far. It is true that frequentist theory does not allow a "state of nature" like B_0 to have a probability distribution, and that Bayesian theory does. However, that does not imply that my usage is Bayesian. (Similarly, although Bayesians view probability as subjective, not all who do so are Bayesians). The point is that there are more than two theories of statistics, although it is often a convenient simplification to characterize the major debate about the fundamentals of statistics as being between two parties: the frequentists and the Bayesians. The fact that this debate remains unresolved is evidence to me that there is not yet one theory of statistics that is universally satisfactory. That being the case, it is the responsibility of the practitioner to decide on what is the appropriate tool for any particular task. Further, it is inevitable that practitioners will, on occasion, differ in their opinions.

The area of confusion in Francis (1992) that I would like to attempt to clarify concerns the interpretation of probability in the definition of a confidence distribution. Walters quite correctly points out that I interpret these distributions in two distinct ways. However, I cannot agree with his description of the frequentist interpretation as "displaying. . .conditional probability". In the frequentist theory, a confidence distribution for B_0 may be thought of as the family of random variables, $\{X_p: p \in (0,1)\}$, with the property that

$$(1) \quad P(X_p > B_0) = p$$

(Kendall and Stuart 1979, section 20.6). Note that this probability statement is unconditional. My departure from frequentist theory did not involve "dropping the conditional term" (as Walters suggests); it involved a second interpretation of (1). The frequentist views (1) as a statement about the proportion of times realizations of the random variable X_p will be greater than the (unknown) constant B_0. I interpreted it both this way and as a statement of my degree of belief that a particular realization of X_p (that derived from the observed data) would be greater than the unknown B_0. The former interpretation is implicit in the procedure for calculating the X_p; the latter is used in the subsequent risk analysis.

From a theoretical point of view, this dual interpretation may perhaps be seen as undesirable. However, a practitioner is more concerned with performance than theoretical elegance. For an evaluation of the performance of the confidence distribution approach in comparison with the (theoretically purer) Bayesian method, I refer the reader to Cordue and Francis (1992) —

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Comment on Meisner (1990): Effect of Climatic Warming on the Southern Margins of the Native Range of Brook Trout, *Salvelinus fontinalis*

Meisner (1990) proposed in the *Journal* that the lower elevational margin of brook trout (*Salvelinus fontinalis*), in the southern part of their native range is related to the 15°C groundwater isotherm, based on a modelled relationship between minimum elevations at which brook trout occur in this part of the native range and elevation, latitude, and groundwater temperature. Furthermore, he estimated minimum elevations for brook trout under the warming scenario of the Goddard Institute for Space Studies (GISS) that projects a 3.8°C increase in mean annual temperature. He presented a crude map of the areas remaining available to brook trout as "potential habitat" under the GISS scenario, but was unable to provide details. He had selected from state inventories the site lowest in each drainage that had brook trout and consequently did not have the entire inventory data set available for the projection. According to Meisner (1990, fig. 3) the distribution of brook trout in North Carolina and Virginia under the GISS scenario would become relatively patchy, and trout would disappear altogether from Tennessee, South Carolina, and Georgia. I was able to apply Meisner's (1990) model to the complete data sets from the North Carolina Wildlife Resources Commission (Bonner 1983) and Virginia Department of Game and Inland Fisheries (Mohn and Bugas 1980) and offer here results and some additional comments.

I extracted from Meisner's (1990) fig. 2 the lower stream boundary under the GISS warming scenario:

$$\text{ELEV} = 7528 - 178.6 \cdot \text{LAT}$$

where latitude is in degrees north and elevation is in metres. The state agencies had assigned elevation to all 1311 streams in the complete inventories and I assigned latitude based on locations on 7.5-min USGS quadrangle sheets. For Virginia streams, I used midpoints of the quadrangle sheets rather than actual latitudes, but the error of using midpoints in the equation above is only 11 m, within the range of error associated with estimating elevations in the inventory database.

Out of 528 streams that had brook trout at the time the inventories were conducted (1978–81 in North Carolina and 1975–79 in Virginia), only 58 would have brook trout under the warming scenario, a loss of 89% of the brook trout streams. A greater loss would occur in Virginia than in North Carolina: in Virginia, only 32 out of 380 brook trout streams would remain, a loss of 92%; and in North Carolina, 26 out of 148 brook trout streams would remain, a loss of 82%. More high-elevation stream habitat is available in North Carolina to harbor brook trout under a warmed climate than is available in Virginia. These results may be optimistic because 32 streams above the minimum elevation specified by the equation above already lack brook trout. In these streams, local stream habitat conditions may be unsuitable or brook trout have already been eliminated

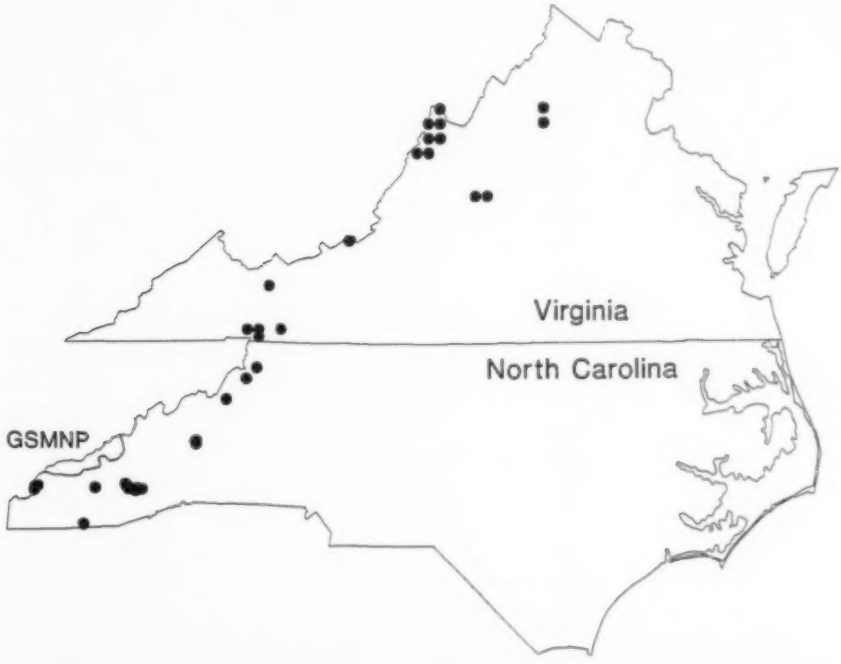

FIG. 1. Location of 58 brook trout streams surveyed in Virginia (1975–79) and North Carolina (1978–81) that would remain above the 15.4°C isotherm under the GISS climate warming scenario, based on the model of Meisner (1990). Each dot may represent more than one stream. The boundary of Great Smoky Mountains National Park (GSMNP) in North Carolina is indicated.

by encroachment by rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). Under global warming, some of these processes may further erode the habitat available for brook trout.

The distribution of these remaining brook trout streams (Fig. 1), although not inconsistent with Meisner's (1990) map, is considerably more fragmented than his map suggests. Part of the fragmentation is because the original inventory represents a sample of all streams, but the sample was sufficiently complete to cover adequately the cold-water stream area of the states. The single exception is that the Great Smoky Mountains National Park along the North Carolina – Tennessee border (see Fig. 1) was not sampled. This high-elevation area should continue to harbor brook trout in some streams under the GISS warming scenario. Other causes of the observed fragmentation are of real concern under a warming scenario. Like all mountainous areas, if only the upper elevations remain available to brook trout, their habitat will take the form of islands on the tops of mountains. At the uppermost elevations, only very small first-order streams remain, and these may be inadequate to support brook trout, particularly under low-flow conditions. In small headwater streams of the Great Smoky Mountains National Park, trout populations occasionally disappear and can only be recolonized from downstream reaches (C.A. Dolloff, USDA Forest Service, Fisheries and Wildlife Department, Virginia Tech, Blacksburg, VA, personal communication), but if these downstream populations are eliminated under warming, these sources of recolonization are not available — Patricia A. Flebbe, *USDA Forest Service, Southeastern Forest Experiment Station and Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0321, USA* (JB617)

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Reply to Flebbe (1993)

Flebbe (1993) has provided a map showing approximate locations of brook trout streams in the native range in Virginia and North Carolina that would remain in the Goddard Institute for Space Studies' (GISS) climate warming scenario (Smith and Tirpak 1989). The streams are located at, or above, the altitude of the lower margin of the native range that I projected would exist in the GISS scenario (Meisner 1990). The lower margin of the southern part of the native range in the GISS scenario, as accurately described by Flebbe's regression of altitude on latitude, reflects the altitude of the 15°C groundwater isotherm, which shapes the lower altitude of brook trout (*Salvelinus fontinalis*) streams in the southern part of the native range (Meisner 1990) such as in Virginia and North Carolina. Flebbe and I used the same stream inventories for Virginia and North Carolina to map the brook trout distributions.

Flebbe's map clarifies the potential effects of climate change on the brook trout distribution in Virginia and North Carolina, based on my groundwater model and the application of the model in the GISS scenario. The distribution of brook trout streams in North Carolina and Virginia in Flebbe's map is more fragmented than the distribution in these states shown in my map (Meisner 1990, Fig. 3) because my map represents an estimate of area of land above the lower distributional boundary, not the locations of brook trout streams. Due to data constraints, as partially restated by Flebbe, I chose to estimate area of the native distribution that would be available to brook trout in a warmer climate. At the time of my study, complete inventories of stream fishes in the native brook trout range were available for some states, but essentially nonexistent for others (e.g., Georgia, South Carolina, and West Virginia).

As a point of correction, I did not state in Meisner (1990) that brook trout habitat would completely disappear in Tennessee in the GISS scenario. On the contrary, as stated in the Results section, Tennessee would probably lose relatively little brook trout habitat. The projected losses for Tennessee are low because most brook trout streams in that state are at altitudes above the projected lower stream boundary in the GISS scenario. A comprehensive inventory of brook trout habitat in Tennessee, including the Great Smoky Mountains National Park, is provided in Bivens (1984).

Flebbe's use of stream inventories for Virginia and North Carolina to identify potential residual brook trout streams in the GISS climate warming scenario points to the existence of other high-altitude, but brook-trout-devoid, streams in the southern part of the native range that may become important refugia for brook trout in a warmer climate. Flebbe reports that 32 of these streams exist for Virginia and North Carolina. Future brook trout rehabilitation/enhancement programs in these states may need to focus efforts on these streams in the advent of global warming. Flebbe's point of the potential development of mountain-top islands of brook trout habitat with climate change is on the mark. The projected brook trout distribution in the GISS scenario in my fig. 3 (Meisner 1990) is a first approximation of these islands. This and Flebbe's map underscore the need to assess the quantity and quality of brook trout and non-brook-trout streams that currently exist in the southern part of the native range at altitudes that would probably be unaffected by climate change. It would be helpful if investigators from the other states of the southern native range followed Flebbe's lead and pooled their stream data to complete the map of brook trout streams remaining in the GISS warming scenario — J. Donald Meisner, *ESSA Environmental and Social Systems Analysts Ltd., 9555 Young Street, No. 308, Richmond Hill, Ont., Canada L4C 9M5*. (JB650)

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